IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Junming Le, Jan Vilcek, Peter Daddona, John Ghrayeb, David Knight and Scott Siegel

Application No.: 10/010,229 Group: 1644

Filed: December 7, 2001 Examiner: Phillip Gambel

Confirmation No.: 8474

For: METHODS OF TREATING MYELODYSPLASTIC SYNDROME WITH ANTI-TNF ANTIBODIES

CERTIFICATE OF MAILING OR TRANSMISSION
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DECLARATION OF JAN VILCEK, M.D.
UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
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Sir:

I, Jan Vilcek, M.D., of 920 5th Avenue New York, NY 10021, U.S.A., declare that:

1. I am a co-inventor of the subject matter described and claimed in the subject application (United States Patent Application Serial No. 10/010,229, filed December 7, 2001). The subject application was filed on behalf of New York University, 550 First Avenue, Rm. MSB153, New

York, NY 10016, U.S.A., and Centocor, Inc., 200 Great Valley Parkway, Malvern, PA, 19355-1307, U.S.A.

2. I received my M.D. degree from Comenius University Medical School, Bratislava, Czechoslovakia in 1957. I received my C.Sc. (Ph.D. equivalent) degree in Virology from the Institute of Virology, Czechoslovak Academy of Science, Bratislava, Czechoslovakia in 1962. A copy of my curriculum vitae, which describes my educational and professional experience, is attached.

3. I have been employed at New York University since 1965. My current position is Professor in the Department of Microbiology.

4. I have read the above-identified patent application and the presently pending claims. I have also read the Office Action dated October 6, 2004.

5. I note that the Examiner stated in the Office Action, dated October 6, 2004, that in order to satisfy the enablement requirement, the cA2 antibody is required to be known and readily available to the public or obtainable by a repeatable method set forth in the specification. I also understand that the deposit of the cell line is not required where the required biological materials can be obtained from publicly available material with only routine experimentation and a reliable screening test. .

The present patent application enables one of skill in the art to carry out the claimed invention and would also enable human and humanized anti-TNF antibodies or antigen-binding fragments thereof. The cA2 antibody is derived from the A2 antibody. The A2 antibody was readily available to the public on the priority date of the instant patent application, March 18, 1991, and was continuously readily available to the public thereafter. On that date, New York University had a general policy of furnishing third parties with a sample of the A2 antibody,

should that third party wish to carry out experiments using the A2 antibody. I have provided antibodies to researchers who requested them. For example, in a letter to me dated March 19, 1992, Dr. Vladimir Lackovic, who was, at the time, at the Institute of Virology in Bratislava (now Slovakia), requested antibodies to tumor necrosis factor (TNF). I replied to Dr. Lackovic in a letter dated April 19, 1992, stating that I sent him monoclonal antibodies to tumor necrosis factor alpha (TNF-α). These antibodies were A2 antibodies. A copy of my letter is attached as Exhibit A. Exhibit B is a translation of Exhibit A. These Exhibits clearly indicate that Applicants made the A2 antibody readily available to the public.

6. I declare that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are believed to be true. Moreover, these statements are made with the knowledge that willful false statements and the like made by me are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon. I attest that I translated Exhibit A from the Slovak language to English and that the translation of the Exhibit is true and accurate.

Jan Vilcek, M.D.

4/5/05
Date

Attachments
Curriculum vitae
Exhibits A and B

EXHIBIT
tabbies
A

NYU Medical Center

Department of Microbiology
550 First Avenue, New York, NY 10016

Tel: (212) 263-5315 Fax: (212) 263 8276

Jan Vilček, M.D.
Professor

New York, 19. 4. 1992

Milý Vlado,

Tvoj list sa nám konečne zatúlal - nebol poslaný leteckou (ste na tom finančne už tak zle, že 1 Kčs rozdiel v poštovnom hrá úlohu?) a kunajší poštári asi mali problém vyluštiť adresu, hoci bola správna!

Dnes sme Ti poslali MAb proti TNF-α. Poslali sme zatiaľ pool média z hybridómových kultúr (nepurifikovaný a nekoncentrovaný). V nezriedenej forme neutralizuje aspoň 5 ng/ml TNF-α a tak na antivirálne pokusy o ktorých píšeš by to malo stačiť. Ak chceš môžeme poslať aj purifikovaný IgG.

Samozrejme, že by som sa tešil možnosti ďalšej spolupráce. Mali by ste záujem pripraviť MAb proti proteínu [redacted] ~~interferónu~~ ktorý sme izolovali v našom laboratóriu? Prikladám informácie o [redacted]. Medzitým sme

ho vyprodukovali v insektových kultúrach a máme ho vypurifikovaný. Viem že existujú mechanizmy ako pre takúto spoluprácu získať peniaze od NIH. Čo si o tom myslíš?

Mnoho pozdravov Tebe a Lacovi.

Srdečne.

Jano

TRANSLATION OF LETTER FROM
JAN VILCEK TO VLADIMIR LACKOVIC
DATED: APRIL 19, 1992

Translated by Dr. Jan Vilcek

Dear Vlado,

Your letter has finally arrived here, it was not sent by air mail (are you so poor that a difference of one Kcs plays a role?) and the postman here probably had a problem deciphering the address even though it was correct!

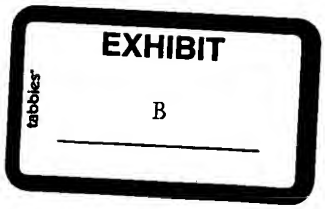
Today we sent you mAbs against TNF-α. We have sent you so far a pool of media from hybridoma cultures (unpurified and unconcentrated). In undiluted form it neutralizes at least 5 ng/ml of TNF-α and so it should be sufficient for the antiviral experiments you mentioned. If you wish we can also send you purified IgG.

Of course, I would welcome a possibility of a further collaboration. Would you be interested in the preparation of mAb against the protein [redacted] which we isolated in our laboratory? I am attaching information about [redacted]. In the meantime, we produced it in insect cultures and we have it purified. I know that there are mechanisms of how to obtain money for such collaboration from the NIH. What do you think about this?

Best regards to you and Laco.

Cordially,

Jan



Myelodysplastic Syndrome

Last Updated: July 30, 2004

Synonyms and related keywords: MDS, preleukemia, refractory dysmyelopoietic anemia, smoldering leukemia, subacute myelogenous leukemia, dysmyelopoiesis, refractory anemia, RA, RA with ringed sideroblasts, RARS, RA with excess blasts, RAEB, RAEB in transformation, RAEB-T, anemia, blood disease, hematopoietic disorders, leukemia, acute leukemia, refractory anemia with ringed sideroblasts, refractory anemia with excess blasts, refractory anemia with excess blasts in transformation, chronic myelomonocytic leukemia, CMML

AUTHOR INFORMATION

Author: **Emmanuel C Besa, MD**, Professor, Department of Internal Medicine, Division of Hematology and Oncology, Drexel University College of Medicine

Coauthor(s): **Ulrich Woermann, MD**, Consulting Staff, Division of Instructional Media, Institute for Medical Education, University of Bern, Switzerland

Emmanuel C Besa, MD, is a member of the following medical societies: American Association for Cancer Education, American Association for the Advancement of Science, American College of Clinical Pharmacology, American Federation for Medical Research, American Society of Clinical Oncology, American Society of Hematology, and New York Academy of Sciences

Editor(s): **Koyamangalath Krishnan, MD, FRCP**, Associate Professor and Research Director, ETSU Cancer Center, Staff Physician, James H. Quillen VA Medical Center, Department of Internal Medicine, East Tennessee State University; **Francisco Talavera, PharmD, PhD**, Senior Pharmacy Editor, Pharmacy, eMedicine; **Troy H Guthrie, Jr, MD**, Chief, Professor, Department of Medicine, Division of Hematology/Oncology, University of Florida School of Medicine; **Rajalaxmi McKenna, MD, FACP**, Southwest Medical Consultants, SC, Department of Medicine, Good Samaritan Hospital, Advocate Health Systems; and **Michael E Zevitz, MD**, Clinical Assistant Professor, Department of Medicine, Finch University of Health Sciences, The Chicago Medical School

INTRODUCTION

Bibliography

Background: Myelodysplastic syndrome (MDS) refers to a heterogeneous group of closely related clonal hematopoietic disorders. All are characterized by a cellular marrow with impaired morphology and maturation (dysmyelopoiesis) and peripheral blood cytopenias, resulting from ineffective blood cell production.

Pathophysiology: The initial hematopoietic stem cell injury can be from cytotoxic chemotherapy, radiation exposure, viral infection, chemical exposure, or genetic predisposition. A clonal mutation predominates over bone marrow, suppressing healthy stem cells.

In early stages, the main cause of cytopenias is increased apoptosis (programmed cell death). As the disease progresses and converts into leukemia, a rare gene mutation occurs and a proliferation of leukemic cells overwhelms the healthy marrow.

Frequency:

- **In the US:** The actual incidence is unknown. MDS was first considered a separate disease in 1976, and occurrence was estimated at 1500 new cases every year. At that time, only patients with less than 5% blasts were considered to have this disorder. The perception that the incidence is increasing may be due to improvements in recognition and criteria for diagnosis. Statistics from 1999 show that 13,000 new cases occur per year (approximately 1000 cases each year in children), surpassing chronic lymphocytic leukemia as the most common form of leukemia in the Western Hemisphere.

- **Internationally:** The disease is found worldwide and is similar in characteristics everywhere.

Mortality/Morbidity: The disease course differs, with some patients having an indolent disease and others having aggressive disease with a very short clinical course that converts into an acute form of leukemia.

Because the diagnostic criteria are new, an international group of hematologists, the French-American-British (FAB) Cooperative Group, classified these disorders into 5 subgroups, differentiating them from acute myeloid leukemia. An underlying trilineage dysplastic change in the bone marrow cells of the patients is found in all subtypes.

- The 2 subgroups of refractory anemia (RA) characterized by 5% or less myeloblasts in bone marrow are (1) RA and (2) RA with ringed sideroblasts (RARS), defined morphologically as having 15% erythroid cells with abnormal ringed sideroblasts, reflecting an abnormal iron accumulation in the mitochondria. Both have a prolonged clinical course and a low prevalence of progression to acute leukemia.

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Patient

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patient

- The 2 subgroups of RAs with greater than 5% myeloblasts are (1) RA with excess blasts (RAEB), defined as 6-20% myeloblasts, and (2) RAEB in transformation (RAEB-T), with 21-30% myeloblasts. The higher the percent of myeloblasts, the shorter the clinical course and the closer the disease is to acute myelogenous leukemia.

  Patient transition from early to more advanced stages indicates these subtypes are merely stages of disease rather than distinct entities. Elderly patients with MDS with trilineage dysplasia and greater than 30% myeloblasts who progress to acute leukemia are often considered to have poor prognoses because their response to chemotherapy is worse than de novo acute myeloid leukemia patients. The 1999 World Health Organization (WHO) classification proposes to include all cases of RAEB-T (patients with >20% myeloblasts) in the category of acute leukemia because these patients have similar prognostic outcomes. However, their response to therapy is worse than patients with the de novo or more typical acute myelogenous leukemia or acute nonlymphocytic leukemia.

- The fifth type of MDS, the most difficult to classify, is called chronic myelomonocytic leukemia (CMML). This subtype can have any percent of myeloblasts but manifests as a monocytosis of 1000/µL or more. It may be associated with splenomegaly. This subtype overlaps with a myeloproliferative disorder (MPD) and may have an intermediate clinical course. CMML must be differentiated from the classic chronic myelocytic leukemia, which is characterized by a negative Ph chromosome. The 1999 WHO classification proposes that juvenile and proliferative CMML be listed separately from the FAB classification under MDS/MPD with splenomegaly and greater than 13,000/µL total WBC count. CMML in the FAB classification under MDS is limited to monocytosis, has less than 13,000/µL total leukocytes, and requires trilineage dysplasia.

Sex: A slight male predominance is noted in all age groups.

Age: MDS primarily affects elderly people, with the median onset in the seventh decade of life.

- The median age of these patients is 65 years, with ages ranging from the early third decade of life to older than 80 years.

- The syndrome may occur in persons of any age group, including the pediatric population.

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History:

- Clinical symptoms that should instigate a workup for MDS are due to low peripheral blood counts, usually from the anemia or thrombocytopenia or neutropenia.
- Fatigue, malaise, and a general feeling of tiredness are due to anemia.
    - Patients with underlying cardiac problems may manifest congestive heart failure, depending on the degree of anemia.
    - Most often, these patients require RBC transfusions to maintain their lifestyle.
- Petechiae, ecchymoses, and nose and gum bleeding are common manifestations of a low platelet count.
    - If underlying dysplastic changes were missed initially, thrombocytopenia as the presenting symptom may be mistaken for immune thrombocytopenia.
    - Poor platelet function is another cause of increased risk of hemorrhage.
- Fever, cough, dysuria, or shock may be manifestations of serious bacterial or fungal infections associated with neutropenia.
- Poor granulocytic function of the existing neutrophils is also attributed to increased risk of infection.

Physical:

- Petechiae or ecchymoses manifest because of bleeding under the skin.
- Epistaxis and gum bleeding are commonly associated with severe thrombocytopenia.
- Hemoptysis, hematuria, and blood in stools may occur.
- Pallor of the skin and mucosal membranes or evidence of fatigue, tachycardia, or congestive heart failure may be manifestations of severe anemia.
- An enlarged spleen may be found in persons with CMML, often indicating an overlap syndrome with an MPD. CMML must to be differentiated from chronic myelocytic leukemia.
- The presence of fever and infections, such as pneumonias and urinary tract infections, may be due to the neutropenia associated with the disease.

Causes:

- Patients who survive malignancy treatment with alkylating agents, with or without radiotherapy, have a high risk of developing MDS or secondary acute leukemia.
- Approximately 60-70% of patients do not have an obvious exposure or cause for MDS and are classified as primary MDS patients.
- Secondary MDS describes the development of MDS or acute leukemia after known exposures to chemotherapy drugs that can cause bone marrow damage. These drugs are associated with a high prevalence of chromosomal abnormalities (following exposure and at the time of MDS or acute leukemia diagnosis).
- Primary, or idiopathic, MDSs are the most common. However, a nonspecific history of exposure to indeterminable chemicals or radiation 10-15 years prior to onset of disease may be present in approximately 50% of patients. This relationship to pathogenesis remains unproved.
    - Other chemicals are leukemogenic.
    - Compounds such as benzene have been implicated.
    - Insecticides, weed killers, and fungicides are possible causes of MDS and secondary leukemia.
    - Less evidence supports genetic predisposition, but familial incidences have been described.
    - Viral infections have also been implicated.

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Agranulocytosis
Anemia
Aplastic Anemia
Bone Marrow Failure
Chronic Myelogenous Leukemia
Hairy Cell Leukemia
Megaloblastic Anemia
Myelophthisic Anemia
Myeloproliferative Disease

Other Problems to be Considered:

Idiopathic thrombocytopenic purpura

Pancytopenia
Thrombocytopenia

Lab Studies:

- Major changes are found in the peripheral blood counts and morphology, and bone marr abnormalities are also present.
- The peripheral blood counts may reflect a single cytopenia (anemia, thrombocytopenia, (neutropenia) in the early phase or bicytopenia (2 deficient cell lines) and pancytopenia (3 cell lines) in later stages.
    - Anemia varies in degree from mild to severe.
    - It is usually macrocytic (mean cell volume of >100 fL) with oval-shaped RBCs (mac ovalocytes).
    - It is usually dimorphic (≥2 populations), consisting of a normal or a hypochromic mi population (RARS) coexisting with the macrocytes.
    - Punctate basophilia is observed in RBCs.
    - Neutropenia may vary from mild to severe.
    - Morphologic abnormalities are often observed in the granulocytes. These can inclu or unsegmented nuclei (pseudo–Pelger-Huet abnormality) or hypersegmentation o nuclei (6-7 lobes) similar to megaloblastic diseases.
    - Granulation abnormalities vary from an absence of granules to abnormal distributic the cytoplasm (Dohle bodies).
    - Platelet counts are decreased (rarely increased) and demonstrate morphologic size abnormalities and cytoplasm abnormalities, such as giant hypogranular platelets ar megakaryocyte fragments.
- In most cases, bone marrow changes include hypercellularity with trilineage dysplastic cl small number of patients may have hypocellular marrow. It often overlaps with aplastic a Increased marrow fibrosis may be confused with other MPDs.
    - Dysplastic changes in RBC lineage (dyserythropoiesis) are characteristic.
    - In the absence of vitamin B-12 or folate deficiencies, it usually manifests similar ch asynchronous maturation of nuclei and cytoplasm as described in megaloblastic ar

- Other changes include binuclearity or multinuclearity in the erythroid cell precursor the presence of ringed sideroblasts (iron accumulation in the mitochondria).
- Dysplastic changes in WBC lineage (dysmyelopoiesis) show myeloid hyperplasia v increased number of myeloblasts and an expanded myelocyte and metamyelocyte (midstage bulge). This separates it from acute leukemia (leukemic hiatus or absen stage).
- Morphologic abnormalities are evident in nuclear-cytoplasm dissociation in maturat when the Pellagroid mature forms are also present in bone marrow.
- Dysthrombopoiesis in the platelet production cell lineage consists of micromegakar (dwarf forms) with poor nuclei lobulation and giant platelets budding off from their c

- Cytogenetic studies of the bone marrow cells indicate mutations into clonal cell lines, witl chromosomes in 48-64% in different series.
- Using higher-resolution techniques (fluorescent in situ hybridization), some practitioners 79% rate of chromosomal abnormalities in primary MDS patients.
  - Chromosomal abnormalities are clonal and include 5q-, monosomy 7 (-7) or 7q-, tri (+8), and numerous other less frequent abnormalities.
  - Multiple combinations may be present, which indicate a very poor prognosis.
  - A single abnormality, except those involving chromosome 7, usually indicates gooc and survival.

Other Tests:

- Cytogenetic techniques have evolved from individual chromosome identification by band techniques to the new, more sensitive color-coded methods.
  - Separating individual chromosomes is dependent on the ability to induce the cell in to identify abnormalities.
  - The new technique using fluorescent in situ hybridization and color-coded chromos enables observation of the intact cell without requiring mitosis.

Histologic Findings: The presence of dysplastic changes in the peripheral blood smear and t dysplasia and hypercellular marrow in the absence of vitamin deficiency is diagnostic of MDS. presence of typical chromosomal abnormalities supports the diagnosis and contributes to dete prognosis.

Staging: Because patients with MDS have heterogenous clinical manifestations and varying c outcomes, staging the patients according to their prognosis and approaching therapy dependi severity and stage is necessary. Also, the FAB classification as discussed previously is not an staging mechanism.

- Recently, an international group of experts convened and determined new criteria for sta the International Prognostic Scoring System (IPSS) for MDS.
  - Table 1. IPSS Score for Staging

Prognostic Variable	0 Points	0.5 Points	1 Point	1.5 Points	2 Points
Bone marrow blasts, %	<5	5-10	...	11-20	21-30
Karyotype*	Good	Intermediate	Poor	...	...
Cytopenias	0/1	2/3	...	...	...

*Good is no abnormality (46,XX or 46,XY), -Y, del(5q), del(20q); intermediate is oth abnormalities, such as trisomy 8 (+8); and poor is complex (33 abnormalities or chi 7 abnormality, ie, 7q- or -7).

  - The first prognostic factor is the amount or percent of myeloblasts in the patient's b marrow study. Each increase of 10% over the reference range is equivalent to half
  - The number of cytopenias is scored by the presence of 2-3 (anemia plus thromboc neutropenia or pancytopenia), which is worth half a point. The presence of none or cytopenia indicates a good prognosis.
  - The total score is added, and the patient is staged according to the following:
    - Low - 0
    - Intermediate 1 - 0.5-1
    - Intermediate 2 - 1.5-2
    - High - Greater than or equal to 2.5
- Classification of the subtypes or categories of MDS has changed from the FAB classifica most recent WHO classification.
  - Table 2. MDS Categories of FAB Classification Versus WHO Classification

FAB	WHO
RA (<5% blasts)	RA Refractory cytopenia with multilineage dysplasia MDS-unclassified MDS with isolated del (5q)
RARS (<5% blasts plus >15% ringed blasts)	RARS Refractory cytopenias with multilineage dysplasia and ringed sideroblasts

RAEB (5-20% blasts)	RAEB-1 (5-9% blasts) RAEB-2 (10-19% blasts)
RAEB-T (21-30% blasts)	Acute myeloid leukemia (>20% blasts)

- CMML in the FAB classification requires an actual monocyte count of more than 1000/µL trilineage dysplasia.
- WHO classifies CMML into the following:
  - Juvenile and proliferative CMML under MDS/MPDs has more than 13,000/µL monc splenomegaly.
  - CMML under MDS is limited to monocytosis of less than 13,000/µL with trilineage c

Medical Care: The standard care for patients with MDS and decreased blood counts is consta changing. Supportive therapy, including transfusions of the cells that are missing (ie, RBCs, pl and treatment of infections are the main treatments. New drugs such as azacitidine (Vidaza) a approved by the US Food and Drug Administration for MDS. Also, new promising drugs such a are completing clinical trials.

Treat the symptoms to improve quality of life. These measures are temporary. More long-term are necessary to stimulate the patient's bone marrow production of mature blood cells.

- Supportive care includes transfusion of RBCs or platelets. The goal is to replace cells tha prematurely undergoing apoptosis in the patient's bone marrow. Guidelines for transfusic patients with MDS and bone marrow failure are as follows:
  - Decrease transfusion-related complications by using leukocyte-depleted blood proc which has been shown to decrease nonhemolytic febrile reactions, prevent alloimm and platelet refractoriness, and prevent cytomegalovirus transmission. Additionally practice has been shown to achieve better quality control of blood products compai bedside filtering and has been shown to be cost effective.
  - Patients with moderate-to-severe anemia require RBC replacement.
    - Transfusing packed RBCs for severe or symptomatic anemia benefits the pat temporarily, only for the life span of the transfused RBCs (2-4 wk).
    - Patients with congestive heart failure may not tolerate the same degree of an young patients with normal cardiac function, and slow or small-volume (eg, pa RBCs) transfusions with judicious use of diuretics should be considered.

        - Consider administering iron chelation for patients receiving 20 or more units c RBCs in order to prevent tissue damage of the liver, heart, pancreas, and oth

    - Platelet transfusion is beneficial to stop active bleeding in thrombocytopenic patien life span for transfused platelets is only 3-7 days.

        - Avoid repeated and frequent platelet transfusions in clinically nonbleeding pa because of low platelet counts (<20,000/µL).

        - Long-term measures to prevent skin and mucosal bleeding may be achieved administering oral antithrombolytic agents such as prophylactic oral epsilon-aminocaproic acid (Amicar) to avoid alloimmunization.

    - Treat infections and neutropenia.

        - Life-threatening infections, especially fungal etiologies, require granulocytes \ antifungal agents.

        - Some patients may require granulocyte transfusions, but the risk of alloimmu high, as is the risk of developing refractoriness to future transfusion therapy.

- Stimulate bone marrow cell production, and decrease excess bone marrow cell apoptosi

    - Hematopoietic growth factors such as erythropoietin (Procrit) for anemia have beer improve anemia in 20% of patients with MDS.

    - Of MDS patients with neutropenia, 75% respond to granulocyte colony-stimulating (Neupogen).

    - Of MDS patients with anemia and neutropenia, 75% respond to a combination of erythropoietin and granulocyte colony-stimulating factor for their neutropenia, with a increase in erythroid response.

- Prevent transition to acute leukemia by administering the newly approved demethylating azacitidine. A pivotal trial in all stages of MDS showed a 37% response (7% complete re 16% partial response) versus a 5% response in the control arm (P <.001) with an improv time to transformation or death (21 mo for azacitidine vs 13 mo for control, P = .001) and transformation to leukemia (15% for azacitidine vs 38% for control, P = .001).

Surgical Care: Splenectomy for the cytopenia associated with MDS is dangerous and fraught complications.

Consultations:

- Patients should be under the care of a hematologist.

- Because most treatment is not standard and is considered experimental, referral to a ter center is often necessary. Encourage patients to participate in clinical trials to determine therapy for this condition.

Treatment is based on the stage and mechanism of the disease that predominates the particul of the disease process. In the early phases, when increased bone marrow apoptosis results in hematopoiesis, retinoids and hematopoietic growth factors are indicated. In late stages, with ir leukemic transformation, cytotoxic chemotherapy and bone marrow transplantation may be ne All of these modes of therapy are undergoing clinical trials to determine the overall benefit to q life and survival.

Cytotoxic chemotherapy is used in MDS patients with increasing myeloblasts and those who h progressed to acute leukemia. The usual combination treatment is a cytarabine-anthracycline combination, which yields a response rate of 30-40% (high complication rate and morbidity in patients).

New drug combinations using hematopoietic growth factors and new drugs, such as topotecar yielding better response rates with lower morbidity. However, these currently are in the early experimental stage.

Drug Category: *Retinoids* -- Most active agents. Vitamin D-3 also has activity but not of clin significant value.

Drug Name	Isotretinoin or 13 *cis*-retinoic acid (Accutane) -- Most active among retinoids. In studies using low doses (20 mg/m^2/d) in a randomized placebo-controlled trial of 70 MDS patients, 1-y survival among RA patients administered drug was 77%, compared with 36% in placebo group. This is statistically significant, although this form of therapy is not generally accepted. The author limits this treatment to patients who are not transfusion dependent.
Adult Dose	Early stage MDS with RA: 20 mg/m^2/d in am pc Author recommends vitamin E 800 mg/d in pm pc to alleviate adverse skin effects
Pediatric Dose	Not established
Contraindications	Documented hypersensitivity
Interactions	Toxicity may occur with vitamin A coadministration; pseudotumor cerebri or papilledema may occur when coadministered with tetracyclines; may reduce plasma levels of carbamazepine
Pregnancy	X - Contraindicated in pregnancy
	Teratogenic and contraindicated in pregnancy and in females of childbearing potential unless they agree to and are capable of mandatory contraceptive

Precautions	measures following a negative result on serum or urine pregnancy test, with a sensitivity of at least 50 IU/mL, within 1 wk prior to beginning therapy; therapy begins on the second or third day of their next menstrual period; repeat pregnancy testing and contraceptive counseling monthly; use sunscreen when outdoors to avoid photosensitization; use skin moisturizers to alleviate skin keratosis

Drug Category: *Hematopoietic growth factors* -- Ineffective blood cell production is due t cellular apoptosis (programmed cell death) caused by activation of the fas-fas ligand. Hematoj growth factors are capable of reversing this process to some extent.

Drug Name	Epoetin alfa (Procrit, Epogen) -- Glycoprotein that stimulates RBC production by stimulating division and maturation of committed RBC precursor cells. Effective in 20-26% of MDS patients when administered alone and in as many as 48% of patients when combined with G-CSF or GM-CSF.
Adult Dose	10,000 U SC 3 times/wk
Pediatric Dose	Not established
Contraindications	Documented hypersensitivity
Interactions	Effectiveness is dependent on availability of iron for heme production; supplementation with 1 $FeSO_4$ tab/d may be sufficient to maintain erythroid response
Pregnancy	C - Safety for use during pregnancy has not been established.
Precautions	Caution in porphyria, hypertension, and history of seizures; decrease dose if hematocrit increase exceeds 4 U in any 2-wk period

Drug Name	Filgrastim (Neupogen) -- G-CSF stimulates division and maturation of granulocytes, mostly neutrophils, in 75-100% of MDS patients and seems to enhance erythroid response in combination with EPO.
Adult Dose	1 mcg/kg/d SC initially; can be adjusted depending on rise of total granulocyte counts; maintenance dose of 300 (in smaller patients) or 480 mcg SC qwk with epoetin alfa is recommended
Pediatric Dose	Not established
Contraindications	Documented hypersensitivity; uncontrolled hypertension
Interactions	None reported
Pregnancy	C - Safety for use during pregnancy has not been established.
	Caution in porphyria, hypertension, and history of

Precautions	seizures; decrease dose if hematocrit increase exceeds 4 U in any 2-wk period; diffuse bone ache or pain may result from stimulation of bone marrow cells

Drug Name	Sargramostim (Leukine) -- GM-CSF stimulates division and maturation of earlier myeloid and macrophage precursor cells. Has been reported to increase granulocytes in 48-91%.
Adult Dose	60-500 mcg/m^2 IV over 2 h to 5-12 mcg/m^2/d SC
Pediatric Dose	Not established
Contraindications	Documented hypersensitivity; excessive myeloid blasts (>10%) in bone marrow or peripheral blood
Interactions	Lithium and corticosteroids may potentiate myeloproliferative effects
Pregnancy	C - Safety for use during pregnancy has not been established.
Precautions	Diffuse bone ache or pain may result from stimulation of bone marrow cells; caution in malignancies with myeloid characteristics

Drug Category: *Demethylation agents* -- Antineoplastics that exert anticancer effects by c DNA demethylation or hypomethylation in abnormal hematopoietic bone marrow cells. May re: normal function to tumor suppressor genes responsible for regulating cell differentiation and gı

Drug Name	Azacitidine (Vidaza) -- Pyrimidine nucleoside analog of cytidine. Interferes with nucleic acid metabolism. Exerts antineoplastic effects by DNA hypomethylation and direct cytotoxicity on abnormal hematopoietic bone marrow cells. Nonproliferative cells are largely insensitive to azacitidine. Indicated to treat MDSs. FDA approved for all 5 MDS subtypes.
Adult Dose	75 mg/m^2 SC qd for 7 d initially, repeat cycle q4wk; may increase to 100 mg/m^2 if no beneficial effect after 2 cycles; treat for a minimum of 4 cycles; treatment may be continued as long as response continues and treatment tolerated
Pediatric Dose	Not established
Contraindications	Documented hypersensitivity to azacitidine or mannitol; advanced malignant hepatic tumors
Interactions	Data limited, none reported
Pregnancy	D - Unsafe in pregnancy
	While on therapy, males should avoid fathering children; do not use during breastfeeding; may cause neutropenia and thrombocytopenia (following first cycle, may require dose adjustment or delay based on nadir counts and hematologic response); caution

Precautions	with hepatic or renal impairment; common adverse effects following SC administration include nausea, vomiting (premedicate for nausea and vomiting before administration), diarrhea, constipation, anemia, thrombocytopenia, leukopenia, neutropenia, pyrexia, fatigue, infection site erythema, and ecchymosis

Further Inpatient Care:

- Bone marrow transplantation with a matched allogeneic or syngeneic donor is used in pa poor prognoses or late-stage MDS who are aged 55 years or younger and have an avail
- Compared with patients with de novo acute myeloid leukemia transplanted in first remiss patients with MDS experience higher mortality rates associated with the procedure (21-3 10%), lower disease-free survival rates, and higher relapse rates (70% vs 40%).
- Because most patients are elderly and only a few young MDS patients will have a match the use of bone marrow transplantation is limited.
- Recently, the use of nonmyeloablative (mini) bone marrow transplantation has been use patients as old as 75 years with some success. This approach is still considered experim should be performed in a clinical trial setting.

Transfer:

- Because most treatment is not standard and is considered experimental, referral to a ter center is often necessary.

Complications:

- The disease itself is associated with complications associated with severe cytopenias. O complications are as follows:
  - The development of myelofibrosis can accelerate a decline in blood counts and an transfusion requirements.
  - Transformation to acute leukemia accelerates the development of complications su anemia, bleeding, and infections.
  - Patients with an enlarged spleen may have complications related to spontaneous r intra-abdominal exsanguination.

Prognosis:

- To improve prognostic classification, the MDS Risk Analysis Workshop has developed th takes into account the cytopenias, percent of bone marrow blasts, and cytogenetics.
    - Those with a good prognoses include patients with single or mild cytopenias, norm chromosomes or a single chromosomal abnormality (except those involving chrom and greater than 10% myeloblasts in the bone marrow. These patients have mean 18-24 months or longer.
    - Patients with pancytopenia requiring RBC or platelet transfusions, patients with chr 7 or multiple abnormalities, and patients with greater than 10% myeloblasts in the b marrow have a 6- to 12-month survival rate.

MISCELLANEOUS

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Medical/Legal Pitfalls:

- If underlying dysplastic changes were missed initially, thrombocytopenia as the presentir symptom may be mistaken for immune thrombocytopenia.
- Splenectomy for the cytopenia in a patient with MDS is dangerous and fraught with comp

PICTURES

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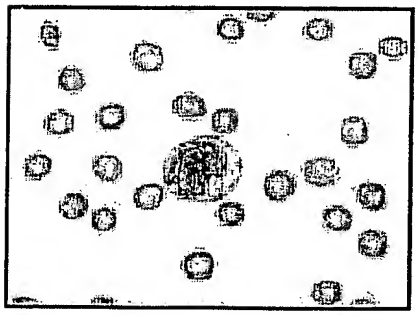
Caption: Picture 1. Myelodysplastic syndrome. Blood film (1000X magnification) demonstrating a vacuolated blast in a refractory anemia with excess of blasts in transformation. Courtesy of U. Woermann, MD, Division of Instructional Media, Institute for Medical Education, University of Bern, Switzerland.

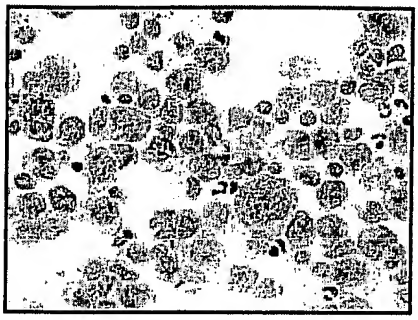


View Full Size Image

eMedicine Zoom View (Interactive!)

Picture Type: Photo

Caption: Picture 2. Myelodysplastic syndrome. This bone marrow film (400X magnification) demonstrates an almost complete replacement of normal hematopoiesis by blast in a refractory anemia with an excess of blasts in transformation. Note the signs of abnormal maturation such as vacuolation, double nucleus, and macrocytosis. Courtesy of U. Woermann, MD, Division of Instructional Media, Institute for Medical Education, University of Bern, Switzerland.

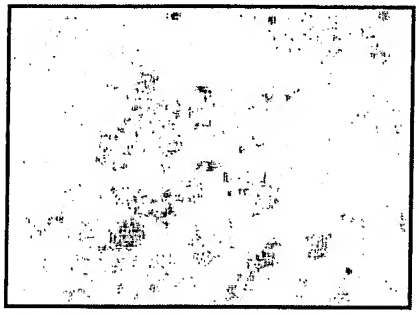


View Full Size Image

eMedicine Zoom View (Interactive!)

Picture Type: Photo

Caption: Picture 3. Myelodysplastic syndrome. Bone marrow film (1000X magnification) demonstrating ring sideroblasts in Prussian blue staining in a refractory anemia with excess of blasts in transformation. Courtesy of U. Woermann, MD, Division of Instructional Media, Institute for Medical Education, University of Bern, Switzerland.

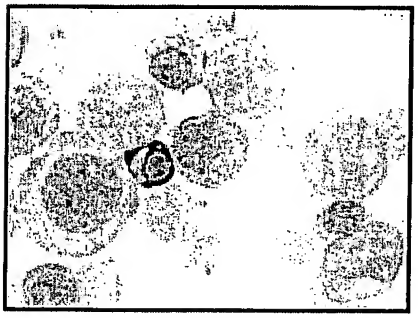


View Full Size Image

eMedicine Zoom View (Interactive!)

Picture Type: Photo

Caption: Picture 4. Myelodysplastic syndrome. Bone marrow film (1000X magnification) demonstrating granular and clotlike positive reaction in periodic acid-Schiff staining in a refractory anemia with excess of blasts in transformation. Courtesy of U. Woermann, MD, Division of Instructional Media, Institute for Medical Education, University of Bern, Switzerland.



View Full Size Image

eMedicine Zoom View (Interactive!)

Picture Type: Photo

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of Law will be entered on the same date herewith.

ORDER AND JUDGMENT

In accordance with the Findings of Fact and Conclusions of Law entered on the same date herewith,

IT IS HEREBY ORDERED AND ADJUDGED, as follows:

1. The Nolan patent (No. 4,506,189), issued on March 19, 1985, is a valid patent.

2. By the manufacture, production, sale and distribution of its SAF-T-COTE fluorescent lamp, Trojan has infringed the Nolan patent.

3. By virtue of this infringement, Shat-R-Shield is entitled to injunctive relief. Trojan shall immediately cease and desist from the manufacture, production, sale and distribution of the SAF-T-COTE fluorescent lamp.

4. Trojan shall recall all the SAF-T-COTE fluorescent lamps sold to and still in the possession of its customers.

5. The Court having determined that Trojan's infringement was not willful and wanton, Shat-R-Shield is not entitled to treble damages.

6. Shat-R-Shield shall have no accounting for monetary damages.

7. The Court having found that this is not an exceptional case, Shat-R-Shield is not entitled to its attorney's fees.

8. All claims having been resolved as to all parties herein, this action is now DISMISSED and STRICKEN from the docket.

9. There being no just reason for delay, this is a FINAL and APPEALABLE Order and Judgment.

Court of Appeals, Federal Circuit

In re Wands

No. 87-1454

Decided September 30, 1988

PATENTS

1. Patentability/Validity — Adequacy of disclosure (§115.12)

Data disclosed in application for immunoassay method patent, which shows that applicants screened nine of 143 cell lines developed for production of antibody necessary to practice invention, stored remainder of said cell lines, and found that four out of nine cell lines screened produced antibody falling within limitation of claims, were erroneously interpreted by Board of Patent Appeals and Interferences as failing to meet disclosure requirements of 35 USC 112, since board's characterization of stored cell lines as "failures" demonstrating unreliability of applicants' methods was improper in view of fact that such unscreened cell lines prove nothing concerning probability of success of person skilled in art attempting to obtain requisite antibodies using applicants' methods.

2. Patentability/Validity — Adequacy of disclosure (§115.12)

Disclosure in application for immunoassay method patent does not fail to meet enablement requirement of 35 USC 112 by requiring "undue experimentation," even though production of monoclonal antibodies necessary to practice invention first requires production and screening of numerous antibody producing cells or "hybridomas," since practitioners of art are prepared to screen negative hybridomas in order to find those that produce desired antibodies, since in monoclonal antibody art one "experiment" is not simply screening of one hybridoma but rather is entire attempt to make desired antibody, and since record indicates that amount of effort needed to obtain desired antibodies is not excessive, in view of applicants' success in each attempt to produce antibody that satisfied all claim limitations.

Appeal from decision of Patent and Trademark Office, Board of Patent Appeals and Interferences.

Application for patent of Jack R. Wands, Vincent R. Zurawski, Jr., and Hubert J. P. Schoemaker, serial number 188,735. From decision of Board of Patent Appeals and Interferences affirming rejection of application, applicants appeal. Reversed; Newman, J., concurring in part and dissenting in part in separate opinion.

Jorge A. Goldstein, of Saidman, Sterne, Kessler & Goldstein (Henry N. Wixon, with them on brief), Washington, D.C., for appellant.

John H. Raubitschek, associate solicitor (Joseph F. Nakamura and Fred E. McKelvey, with him on brief), PTO, for appellee.

Before Smith, Newman, and Bissell, circuit judges.

Smith, J.

This appeal is from the decision of the Patent and Trademark Office (PTO) Board of Patent Appeals and Interferences (board) affirming the rejection of all remaining claims in appellant's application for a patent, serial No. 188,735, entitled "Immunoassay Utilizing Monoclonal High Affinity IgM

Antibodies," which was filed September 19, 1980.[1] The rejection under 35 U.S.C. §112, first paragraph, is based on the grounds that appellant's written specification would not enable a person skilled in the art to make the monoclonal antibodies that are needed to practice the claimed invention without undue experimentation. We reverse.

I. *Issue*

The only issue on appeal is whether the board erred, as a matter of law, by sustaining the examiner's rejection for lack of enablement under 35 U.S.C. §112, first paragraph, of all remaining claims in appellants' patent application, serial No. 188,735.

II. *Background*

A. *The Art.*

The claimed invention involves immunoassay methods for the detection of hepatitis B surface antigen by using high-affinity monoclonal antibodies of the IgM isotype. *Antibodies* are a class of proteins (immunoglobulins) that help defend the body against invaders such as viruses and bacteria. An antibody has the potential to bind tightly to another molecule, which molecule is called an antigen. The body has the ability to make millions of different antibodies that bind to different antigens. However, it is only after exposure of an antigen that a complicated *immune response* leads to the production of antibodies against that antigen. For example, on the surface of hepatitis B virus particles there is a large protein called *hepatitis B surface antigen* (HBsAg). As its name implies, it is capable of serving as an antigen. During a hepatitis B infection (or when purified HBsAg is injected experimentally), the body begins to make antibodies that bind tightly and specifically to HBsAg. Such antibodies can be used as reagents for sensitive diagnostic tests (*e.g.*, to detect hepatitis B virus in blood and other tissues, a purpose of the claimed invention). A method for detecting or measuring antigens by using antibodies as reagents is called an *immunoassay*.

Normally, many different antibodies are produced against each antigen. One reason for this diversity is that different antibodies are produced that bind to different regions (determinants) of a large antigen molecule such as HBsAg. In addition, different antibodies may be produced that bind to the same determinant. These usually differ in the tightness with which they bind to the determinant. *Affinity* is a quantitative measure of the strength of antibody-antigen binding. Usually an antibody with a higher affinity for an antigen will be more useful for immunological diagnostic tests than one with a lower affinity. Another source of heterogeneity is that there are several immunoglobulin classes or *isotypes*. Immunoglobulin G (IgG) is the most common isotype in serum. Another isotype, immunoglobulin M (IgM), is prominent early in the immune response. IgM molecules are larger than IgG molecules, and have 10 antigen-binding sites instead of the 2 that are present in IgG. Most immunoassay methods use IgG, but the claimed invention uses only IgM antibodies.

For commercial applications there are many disadvantages to using antibodies from serum. Serum contains a complex mixture of antibodies against the antigen of interest within a much larger pool of antibodies directed at other antigens. There are available only in a limited supply that ends when the donor dies. The goal of monoclonal antibody technology is to produce an unlimited supply of a single purified antibody.

The blood cells that make antibodies are *lymphocytes*. Each lymphocyte makes only one kind of antibody. During an immune response, lymphocytes exposed to their particular antigen divide and mature. Each produces a *clone* of identical daughter cells, all of which secrete the same antibody. Clones of lymphocytes, all derived from a single lymphocyte, could provide a source of a single homogeneous antibody. However, lymphocytes do not survive for long outside of the body in cell culture.

Hybridoma technology provides a way to obtain large numbers of cells that all produce the same antibody. This method takes advantage of the properties of *myeloma* cells derived from a tumor of the immune system. The cancerous myeloma cells can divide indefinitely in vitro. They also have the potential ability to secrete antibodies. By appropriate experimental manipulations, a myeloma cell can be made to fuse with a lymphocyte to produce a single hybrid cell (hence, a hybridoma) that contains the genetic material of both cells. The hybridoma secretes the same antibody that was made by its parent lymphocyte, but acquires the capability of the myeloma cell to divide and grow indefinitely in cell culture. Antibodies produced by a clone of hybridoma cells (i.e., by hybridoma

[1] *In re Wands*, Appeal No. 673-76 (Bd. Pat. App. & Int. Dec. 30, 1986).

cells that are all progeny of a single cell) are called monoclonal antibodies.[2]

B. *The Claimed Invention.*

The claimed invention involves methods for the immunoassay of HBsAg by using high-affinity monoclonal IgM antibodies. Jack R. Wands and Vincent R. Zurawski, Jr., two of the three coinventors of the present application, disclosed methods for producing monoclonal antibodies against HBsAg in United States patent No. 4,271,145 (the '145 patent), entitled "Process for Producing Antibodies to Hepatitis Virus and Cell Lines Therefor," which patent issued on June 2, 1981. The '145 patent is incorporated by reference into the application on appeal. The specification of the '145 patent teaches a procedure for immunizing mice against HBsAg, and the use of lymphocytes from these mice to produce hybridomas that secrete monoclonal antibodies specific for HBsAg. The '145 patent discloses that this procedure yields both IgG and IgM antibodies with high-affinity binding to HBsAg. For the stated purpose of complying with the best mode requirement of 35 U.S.C. §112, first paragraph, a hybridoma cell line that secretes IgM antibodies against HBsAg (the 1F8 cell line) was deposited at the American Type Culture Collection, a recognized cell depository, and became available to the public when the '145 patent issued.

The application on appeal claims methods for immunoassay of HBsAg using monoclonal antibodies such as those described in the '145 patent. Most immunoassay methods have used monoclonal antibodies of the IgG isotype. IgM antibodies were disfavored in the prior art because of their sensitivity to reducing agents and their tendency to self-aggregate and precipitate. Appellants found that their monoclonal IgM antibodies could be used for immunoassay of HbsAg with unexpectedly high sensitivity and specificity. Claims 1, 3, 7, 8, 14, and 15 are drawn to methods for the immunoassay of HBsAg using high-affinity IgM monoclonal antibodies. Claims 19 and 25–27 are for chemically modified (*e.g.*, radioactively labeled) monoclonal IgM antibodies used in the assays. The broadest method claim reads:

> 1. An immunoassay method utilizing an antibody to assay for a substance comprising hepatitis B-surface antigen (HBsAg) determinants which comprises the steps of:
>
> contacting a test sample containing said substance comprising HBsAg determinants with said antibody; and
>
> determining the presence of said substance in said sample;
>
> wherein said antibody is a monoclonal high affinity IgM antibody having a binding affinity constant for said HBsAg determinants of at least 10^9 M^{-1}.

Certain claims were rejected under 35 U.S.C. §103; these rejections have not been appealed. Remaining claims 1, 3, 7, 8, 14, 15, 19, and 25–27 were rejected under 35 U.S.C. §112, first paragraph, on the grounds that the disclosure would not enable a person skilled in the art to make and use the invention without undue experimentation. The rejection is directed solely to whether the specification enables one skilled in the art to make the monoclonal antibodies that are needed to practice the invention. The position of the PTO is that data presented by Wands show that the production of high-affinity IgM anti-HBsAg antibodies is unpredictable and unreliable, so that it would require undue experimentation for one skilled in the art to make the antibodies.

III. *Analysis*

A. *Enablement by Deposit of Micro-organisms and Cell Lines.*

The first paragraph of 35 U.S.C. §112 requires that the specification of a patent must enable a person skilled in the art to make and use the claimed invention. "Patents * * * are written to enable those skilled in the art to practice the invention."[3] A patent need not disclose what is well known in the art.[4] Although we review underlying facts found by the board under a "clearly erroneous" standard,[5] we review enablement as a question of law.[6]

Where an invention depends on the use of living materials such as microorganisms or

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[2] For a concise description of monoclonal antibodies and their use in immunoassay see *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1368–71, 231 USPQ 81, 82–83 (Fed. Cir. 1986), *cert. denied*, 107 S.Ct. 1606 (1987).

[3] *W.L. Gore & Assocs., Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1556, 220 USPQ 303, 315 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984).

[4] *Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.*, 730 F.2d 1452, 1463, 221 USPQ 481, 489 (Fed. Cir. 1984).

[5] *Coleman v. Dines*, 754 F.2d 353, 356, 224 USPQ 857, 859 (Fed. Cir. 1985).

[6] *Moleculon Research Corp. v. CBS, Inc.*, 793 F.2d 1261, 1268, 229 USPQ 805, 810 (Fed. Cir. 1986), *cert. denied*, 107 S.Ct. 875 (1987); *Raytheon Co. v. Roper Corp.*, 724 F.2d 951, 960 n.6, 220 USPQ 592, 599 n.6 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 835 [225 USPQ 232] (1984).

cultured cells, it may be impossible to enable the public to make the invention (i.e., to obtain these living materials) solely by means of a written disclosure. One means that has been developed for complying with the enablement requirement is to deposit the living materials in cell depositories which will distribute samples to the public who wish to practice the invention after the patent issues.[7] Administrative guidelines and judicial decisions have clarified the conditions under which a deposit of organisms can satisfy the requirements of section 112.[8] A deposit has been held necessary for enablement where the starting materials (i.e., the living cells used to practice the invention, or cells from which the required cells can be produced) are not readily available to the public.[9] Even when starting materials are available, a deposit has been necessary where it would require undue experimentation to make the cells of the invention from the starting materials.[10]

In addition to satisfying the enablement requirement, deposit of organisms also can be used to establish the filing date of the application as the prima facie date of invention,[11] and to satisfy the requirement under 35 U.S.C. §114 that the PTO be guaranteed access to the invention during pendency of the application.[12] Although a deposit may serve these purposes, we recognized, in *In re Lundak*,[13] that these purposes, nevertheless, may be met in ways other than by making a deposit.

A deposit also may satisfy the best mode requirement of section 112, first paragraph, and it is for this reason that the 1F8 hybridoma was deposited in connection with the '145 patent and the current application. Wands does not challenge the statements by the examiner to the effect that, although the deposited 1F8 line enables the public to perform immunoassays with antibodies produced by that single hybridoma, the deposit does not enable the generic claims that are on appeal. The examiner rejected the claims on the grounds that the written disclosure was not enabling and that the deposit was inadequate. Since we hold that the written disclosure fully enables the claimed invention, we need not reach the question of the adequacy of deposits.

B. *Undue Experimentation.*

Although inventions involving microorganisms or other living cells often can be enabled by a deposit,[14] a deposit is not always necessary to satisfy the enablement requirement.[15] No deposit is necessary if the biological organisms can be obtained from readily available sources or derived from readily available starting materials through routine screening that does not require undue experimentation.[16] Whether the specification in an application involving living cells (here, hybridomas) is enabled without a deposit must be decided on the facts of the particular case.[17]

Appellants contend that their written specification fully enables the practice of

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[7] *In re Argoudelis*, 434 F.2d 1390, 1392–93, 168 USPQ 99, 101–02 (CCPA 1970).

[8] *In re Lundak*, 773 F.2d 1216, 227 USPQ 90 (Fed. Cir. 1985); *Feldman v. Aunstrup*, 517 F.2d 1351, 186 USPQ 108 (CCPA 1975), *cert. denied*, 424 U.S. 912 [188 USPQ 720] (1976); Manual of Patent Examining Procedure (MPEP) 608.01 (p)(C) (5th ed. 1983, rev. 1987). *See generally* Hampar, *Patenting of Recombinant DNA Technology: The Deposit Requirement*, 67 J. Pat. Trademark Off. Soc'y 569 (1985).

[9] *In re Jackson*, 217 USPQ 804, 807–08 (Bd. App. 1982) (strains of a newly discovered species of bacteria isolated from nature); *Feldman*, 517 F.2d 1351, 186 USPQ 108 (uncommon fungus isolated from nature); *In re Argoudelis*, 434 F.2d at 1392, 168 USPQ at 102 (novel strain of antibiotic-producing microorganism isolated from nature); *In re Kropp*, 143 USPQ 148, 152 (Bd. App. 1959) (newly discovered microorganism isolated from soil).

[10] *Ex parte Forman*, 230 USPQ 546, 547 (Bd. Pat. App. & Int. 1986) (genetically engineered bacteria where the specification provided insufficient information about the amount of time and effort required); *In re Lundak*, 773 F.2d 1216, 227 USPQ 90 (unique cell line produced from another cell line by mutagenesis).

[11] *In re Lundak*, 773 F.2d at 1222, 227 USPQ at 95–96; *In re Feldman*, 517 F.2d at 1355, 186 USPQ at 113; *In re Argoudelis*, 434 F.2d at 1394–96, 168 USPQ at 103–04 (Baldwin, J. concurring).

[12] *In re Lundak*, 773 F.2d at 1222, 227 USPQ at 95–96; *In re Feldman*, 517 F.2d at 1354, 186 USPQ at 112.

[13] *In re Lundak*, 773 F.2d at 1222, 227 USPQ at 95–96.

[14] *In re Argoudelis*, 434 F.2d at 1393, 168 USPQ at 102.

[15] *Tabuchi v. Nubel*, 559 F.2d 1183, 194 USPQ 521 (CCPA 1977).

[16] *Id.* at 1186–87, 194 USPQ at 525; *Merck & Co. v. Chase Chem. Co.*, 273 F.Supp. 68, 77, 155 USPQ 139, 146 (D.N.J. 1967); *Guaranty Trust Co. v. Union Solvents Corp.*, 54 F.2d 400, 403–06, 12 USPQ 47, 50–53 (D. Del. 1931), *aff'd*, 61 F.2d 1041, 15 USPQ 237 (3d Cir. 1932), *cert. denied*, 288 U.S. 614 (1933); MPEP 608.01(p)(C) ("No problem exists when the microorganisms used are known and readily available to the public.").

[17] *In re Jackson*, 217 USPQ at 807; *see In re Metcalfe*, 410 F.2d 1378, 1382, 161 USPQ 789, 792 (CCPA 1969).

their claimed invention because the monoclonal antibodies needed to perform the immunoassays can be made from readily available starting materials using methods that are well known in the monoclonal antibody art. Wands states that application of these methods to make high-affinity IgM anti-HBsAg antibodies requires only routine screening, and that does not amount to undue experimentation. There is no challenge to their contention that the starting materials (i.e., mice, HBsAg antigen, and myeloma cells) are available to the public. The PTO concedes that the methods used to prepare hybridomas and to screen them for high-affinity IgM antibodies against HBsAg were either well known in the monoclonal antibody art or adequately disclosed in the '145 patent and in the current application. This is consistent with this court's recognition with respect to another patent application that methods for obtaining and screening monoclonal antibodies were well known in 1980.[18] The sole issue is whether, in this particular case, it would require undue experimentation to produce high-affinity IgM monoclonal antibodies.

Enablement is not precluded by the necessity for some experimentation such as routine screening.[19] However, experimentation needed to practice the invention must not be undue experimentation.[20] "the key word is 'undue,' not 'experimentation.' "[21]

> The determination of what constitutes undue experimentation in a given case requires the application of a standard of reasonableness, having due regard for the nature of the invention and the state of the art. Ansul Co. v. Uniroyal, Inc. [448 F.2d 872, 878-79; 169 USPQ 759, 762-63 (2d Cir. 1971), *cert. denied*, 404 U.S. 1018 [172 USPQ 257] (1972)]. The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed * * * .[22]

The term "undue experimentation" does not appear in the statute, but it is well established that enablement requires that the specification teach those in the art to make and use the invention without undue experimentation.[23] Whether undue experimentation is needed is not a single, simple factual determination, but rather is a conclusion reached by weighing many factual considerations. The board concluded that undue experimentation would be needed to practice the invention on the basis of experimental data presented by Wands. These data are not in dispute. However, Wands and the board disagree strongly on the conclusion that should be drawn from that data.

Factors to be considered in determining whether a disclosure would require undue experimentation have been summarized by the board in *Ex parte Forman*.[24] They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims.[25]

In order to understand whether the rejection was proper, it is necessary to discuss further the methods for making specific monoclonal antibodies. The first step for making monoclonal antibodies is to immunize an animal. The '145 patent provides a detailed description of procedures for immunizing a specific strain of mice against HBsAg. Next the spleen, an organ rich in lymphocytes, is removed and the lymphocytes are separated from the other spleen cells. The lymphocytes are mixed with myeloma cells, and the mixture is treated to cause a few of the cells to fuse with each other. Hybridoma cells that secrete the desired antibodies then must be isolated from the enormous number of other cells in the mixture. This is done through a series of screening procedures.

The first step is to separate the hybridoma cells from unfused lymphocytes and myeloma cells. The cells are cultured in a medi-

[18] *Hybritech*, 802 F.2d at 1384, 231 USPQ at 94.

[19] *Id.; Atlas Powder Co. v. E.I. DuPont De Nemours & Co.*, 750 F.2d 1569, 1576, 224 USPQ 409, 413 (Fed. Cir. 1984); *In re Angstadt*, 537 F.2d at 502-504, 190 USPQ at 218; *In re Geerdes*, 491 F.2d 1260, 1265, 180 USPQ 789, 793 (CCPA 1974); *Mineral Separation, Ltd. v. Hyde*, 242 U.S. 261, 270-71 (1916).

[20] *Hybritech*, 802 F.2d at 1384, 231 USPQ at 94; *W.L. Gore*, 721 F.2d at 1557, 220 USPQ at 316; *In re Colianni*, 561 F.2d 220, 224, 195 USPQ 150, 153 (CCPA 1977) (Miller, J., concurring).

[21] *In re Angstadt*, 537 F.2d at 504, 190 USPQ at 219.

[22] *In re Jackson*, 217 USPQ at 807.

[23] *See Hybritech*, 802 F.2d at 1384, 231 USPQ at 94; *Atlas Powder*, 750 F.2d at 1576, 224 USPQ at 413.

[24] *Ex parte Forman*, 230 USPQ at 547.

[25] *Id.; see In re Colianni*, 561 F.2d at 224, 195 USPQ at 153 (Miller, J., concurring); *In re Rainer*, 347 F.2d 574, 577, 146 USPQ 218, 221 (CCPA 1965).

um in which all the lymphocytes and myeloma cells die, and only the hybridoma cells survive. The next step is to isolate and clone hybridomas that make antibodies that bind to the antigen of interest. Single hybridoma cells are placed in separate chambers and are allowed to grow and divide. After there are enough cells in the clone to produce sufficient quantities of antibody to analyze, the antibody is assayed to determine whether it binds to the antigen. Generally, antibodies from many clones do not bind the antigen, and these clones are discarded. However, by screening enough clones (often hundreds at a time), hybridomas may be found that secrete antibodies against the antigen of interest.

Wands used a commercially available radioimmunoassay kit to screen clones for cells that produce antibodies directed against HBsAg. In this assay the amount of radioactivity bound gives some indication of the strength of the antibody-antigen binding, but does not yield a numerical affinity constant, which must be measured using the more laborious Scatchard analysis. In order to determine which anti-HBsAg antibodies satisfy all of the limitations of appellants' claims, the antibodies require further screening to select those which have an IgM isotype and have a binding affinity constant of at least 10^9 M^{-1}.[26] The PTO does not question that the screening techniques used by Wands were well known in the monoclonal antibody art.

During prosecution Wands submitted a declaration under 37 C.F.R §1.132 providing information about all of the hybridomas that appellants had produced before filing the patent application. The first four fusions were unsuccessful and produced no hybridomas. The next six fusion experiments all produced hybridomas that made antibodies specific for HBsAg. Antibodies that bound at least 10,000 cpm in the commercial radioimmunoassay were classified as "high binders." Using this criterion, 143 high-binding hybridomas were obtained. In the declaration, Wands stated that [27]

> It is generally accepted in the art that, among those antibodies which are binders with 50,000 cpm or higher, there is a very high likelihood that high affinity (Ka [greater than] 10^9 M^{-1}) antibodies will be found. However, high affinity antibodies can also be found among high binders of between 10,000 and 50,000, as is clearly demonstrated in the Table.

The PTO has not challenged this statement.

The declaration stated that a few of the high-binding monoclonal antibodies from two fusions were chosen for further screening. The remainder of the antibodies and the hybridomas that produced them were saved by freezing. Only nine antibodies were subjected to further analysis. Four (three from one fusion and one from another fusion) fell within the claims, that is, were IgM antibodies and had a binding affinity constant of at least 10^9 M^{-1}. Of the remaining five antibodies, three were found to be IgG, while the other two were IgM for which the affinity constants were not measured (although both showed binding well above 50,000 cpm).

Apparently none of the frozen cell lines received any further analysis. The declaration explains that after useful high-affinity IgM monoclonal antibodies to HBsAg had been found, it was considered unnecessary to return to the stored antibodies to screen for more IgMs. Wands says that the existence of the stored hybridomas was disclosed to the PTO to comply with the requirement under 37 C.F.R. §1.56 that applicants fully disclose all of their relevant data, and not just favorable results.[28] How these stored hybridomas are viewed is central to the positions of the parties.

The position of the board emphasizes the fact that since the stored cell lines were not completely tested, there is no proof that any of them are IgM antibodies with a binding affinity constant of at least 10^9 M^{-1}. Thus, only 4 out of 143 hybridomas, or 2.8 percent, were *proved* to fall within the claims. Furthermore, antibodies that were proved to be high-affinity IgM came from only 2 of 10 fusion experiments. These statistics are viewed by the board as evidence that appellants' methods were not predictable or reproducible. The board concludes that Wands' low rate of demonstrated success shows that a person skilled in the art would have to

[26] The examiner, the board, and Wands all point out that, technically, the strength of antibody-HBsAg binding is measured as *avidity*, which takes into account multiple determinants on the HBsAg molecule, rather than affinity. Nevertheless, despite this correction, all parties then continued to use the term "affinity." We will use the terminology of the parties. Following the usage of the parties, we will also use the term "high-affinity" as essentially synonymous with "having a binding affinity constant of at least 10^9 M^{-1}."

[27] A table in the declaration presented the binding data for antibodies from every cell line. Values ranged from 13,867 to 125,204 cpm, and a substantial proportion of the antibodies showed binding greater than 50,000 cpm. In confirmation of Dr. Wand's statement, two antibodies with binding less than 25,000 cpm were found to have affinity constants greater than 10^9 M^{-1}.

[28] *See Rohm & Haas Co. v. Crystal Chem. Co.*, 722 F.2d 1556, 220 USQ 98 (Fed. Cir. 1983).

engage in undue experimentation in order to make antibodies that fall within the claims.

Wands views the data quite differently. Only nine hybridomas were actually analyzed beyond the initial screening for HBsAg binding. Of these, four produced antibodies that fell within the claims, a respectable 44 percent rate of success. (Furthermore, since the two additional IgM antibodies for which the affinity constants were never measured showed binding in excess of 50,000 cpm, it is likely that these also fall within the claims.) Wands argues that the remaining 134 unanalyzed, stored cell lines should not be written off as failures. Instead, if anything, they represent partial success. Each of the stored hybridomas had been shown to produce a high-binding antibody specific for HBsAg. Many of these antibodies showed binding above 50,000 cpm and are thus highly likely to have a binding affinity constant of at least 10^9 M^{-1}. Extrapolating from the nine hybridomas that were screened for isotype (and from what is well known in the monoclonal antibody art about isotype frequency), it is reasonable to assume that the stored cells include some that produce IgM. Thus, if the 134 incompletely analyzed cell lines are considered at all, they provide some support (albeit without rigorous proof) to the view that hybridomas falling within the claims are not so rare that undue experimentation would be needed to make them.

The first four fusion attempts were failures, while high-binding antibodies were produced in the next six fusions. Appellants contend that the initial failures occurred because they had not yet learned to fuse cells successfully. Once they became skilled in the art, they invariably obtained numerous hybridomas that made high-binding antibodies against HBsAg and, in each fusion where they determined isotype and binding affinity they obtained hybridomas that fell within the claims.

Wands also submitted a second declaration under 37 C.F.R. §1.132 stating that after the patent application was submitted they performed an eleventh fusion experiment and obtained another hybridoma that made a high-affinity IgM anti-HBsAg antibody. No information was provided about the number of clones screened in that experiment. The board determined that, because there was no indication as to the number of hybridomas screened, this declaration had very little value. While we agree that it would have been preferable if Wands had included this information, the declaration does show that when appellants repeated their procedures they again obtained a hybridoma that produced an antibody that fit all of the limitations of their claims.

[1] We conclude that the board's interpretation of the data is erroneous. It is strained and unduly harsh to classify the stored cell lines (each of which was proved to make high-binding antibodies against HBsAg) as failures demonstrating that Wands' methods are unpredictable or unreliable.[29] At worst, they prove nothing at all about the probability of success, and merely show that appellants were prudent in not discarding cells that might someday prove useful. At best, they show that high-binding antibodies, the starting materials for IgM screening and Scatchard analysis, can be produced in large numbers. The PTO's position leads to the absurd conclusion that the more hybridomas an applicant makes and saves without testing the less predictable the applicant's results become. Furthermore, Wands' explanation that the first four attempts at cell fusion failed only because they had not yet learned to perform fusions properly is reasonable in view of the fact that the next six fusions were all successful. The record indicates that cell fusion is a technique that is well known to those of ordinary skill in the monoclonal antibody art, and there has been no claim that the fusion step should be more difficult or unreliable where the antigen is HBsAg than it would be for other antigens.

[2] When Wands' data is interpreted in a reasonable manner, analysis considering the factors enumerated in *Ex parte Forman* leads to the conclusion that undue experimentation would not be required to practice the invention. Wands' disclosure provides considerable direction and guidance on how to practice their invention and presents working examples. There was a high level of skill in the art at the time when the application was filed, and all of the methods needed to practice the invention were well known.

The nature of monoclonal antibody technology is that it involves screening hybridomas to determine which ones secrete antibody with desired characteristics. Practitioners of this art are prepared to screen negative hybridomas in order to find one that makes the desired antibody. No evidence was presented by either party on how many hybridomas would be viewed by those in the art as requiring undue experimentation to screen. However, it seems unlikely that un-

[29] Even if we were to accept the PTO's 2.8% success rate, we would not be required to reach a conclusion of undue experimentation. Such a determination must be made in view of the circumstances of each case and cannot be made solely by reference to a particular numerical cutoff.

due experimentation would be defined in terms of the number of hybridomas that were never screened. Furthermore, in the monoclonal antibody art it appears that an "experiment" is not simply the screening of a single hybridoma, but is rather the entire attempt to make a monoclonal antibody against a particular antigen. This process entails immunizing animals, fusing lymphocytes from the immunized animals with myeloma cells to make hybridomas, cloning the hybridomas, and screening the antibodies produced by the hybridomas for the desired characteristics. Wands carried out this entire procedure three times, and was successful each time in making at least one antibody that satisfied all of the claim limitations. Reasonably interpreted, Wands' record indicates that, in the production of high-affinity IgM antibodies against HBsAG, the amount of effort needed to obtain such antibodies is not excessive. Wands' evidence thus effectively rebuts the examiner's challenge to the enablement of their disclosure.[30]

IV. *Conclusion*

Considering all of the factors, we conclude that it would not require undue experimentation to obtain antibodies needed to practice the claimed invention. Accordingly, the rejection of Wands' claims for lack of enablement under 35 U.S.C. §112, first paragraph, is reversed.

REVERSED

Newman, J., concurring in part, dissenting in part.

A

I concur in the court's holding that additional samples of hybridoma cell lines that produce these high-affinity IgM monoclonal antibodies need not be deposited. This invention, as described by Wands, is not a selection of a few rare cells from many possible cells. To the contrary, Wands states that all monoclonally produced IgM antibodies to hepatitis B surface antigen have the desired high avidity and other favorable properties, and that all are readily preparable by now-standard techniques.

Wands states that his United States Patent No. 4,271,145 describes fully operable techniques, and is distinguished from his first four failed experiments that are referred to in the Rule 132 affidavit. Wands argues that these biotechnological mechanisms are relatively well understood and that the preparations can be routinely duplicated by those of skill in this art, as in *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1380, 231 USPQ 81, 94 (Fed. Cir. 1986), *cert. denied*, 107 S.Ct. 1606 (1987). I agree that it is not necessary that there be a deposit of multiple exemplars of a cell system that is readily reproduced by known, specifically identified techniques.

B

I would affirm the board's holding that Wands has not complied with 35 U.S.C. §112, first paragraph, in that he has not provided data sufficient to support the breadth of his generic claims. Wands' claims on appeal include the following:

> 19. Monoclonal high affinity IgM antibodies immunoreactive with HBsAg determinants, wherein said antibodies are coupled to an insoluble solid phase, and wherein the binding affinity constant of said antibodies for said HBsAg determinants is at least 10^9 M^{-1}.
>
> 26. Monoclonal high affinity IgM antibodies immunoreactive with hepatitis B surface antigen.

Wands states that he obtained 143 "high binding monoclonal antibodies of the right specificity" in the successful fusions; although he does not state how they were determined to be high binding or of the right specificity, for Wands also states that only nine of these 143 were tested.

Of these nine, four (three from one fusion and one from another fusion) were found to have the claimed high affinity and to be of the IgM isotype. Wands states that the other five were either of a different isotype or their affinities were not determined. (This latter statement also appears to contradict his statement that all 143 were "high binding".)

Wands argues that a "success rate of four out of nine", or 44.4%, is sufficient to support claims to the entire class. The Commissioner deems the success rate to be four out of 143, or 2.8%; to which Wands responds with statistical analysis as to how unlikely it is that Wands selected the only four out of 143 that worked. Wands did not, however, prove the right point. The question is whether Wands, by testing nine out of 143 (the Commissioner points out that the randomness of the sample was not established), and finding that four out of the nine had the desired properties, has provided sufficient experimental support for the breadth of the requested claims, in the context that "experi-

[30] *In re Strahilevitz*, 668 F.2d 1229, 1232, 212 USPQ 561, 563 (CCPA 1982).

ments in genetic engineering produce, at best, unpredictable results", quoting from *Ex parte Forman*, 230 USPQ 546, 547 (Bd.Pat.App. and Int. 1986).

The premise of the patent system is that an inventor, having taught the world something it didn't know, is encouraged to make the product available for public and commercial benefit, by governmental grant of the right to exclude others from practice of that which the inventor has disclosed. The boundary defining the excludable subject matter must be carefully set: it must protect the inventor, so that commercial development is encouraged; but the claims must be commensurate with the inventor's contribution. Thus the specification and claims must meet the requirements of 35 U.S.C. §112. *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 23-24 (CCPA 1970).

As the science of biotechnology matures the need for special accommodation, such as the deposit of cell lines or microorganisms, may diminish; but there remains the body of law and practice on the need for sufficient disclosure, including experimental data when appropriate, that reasonably support the scope of the requested claims. That law relates to the sufficiency of the description of the claimed invention, and if not satisfied by deposit, must independently meet the requirements of Section 112.

Wands is not claiming a particular, specified IgM antibody. He is claiming all such monoclonal antibodies in assay for hepatitis B surface antigen, based on his teaching that such antibodies have uniformly reproducible high avidity, free of the known disadvantages of IgM antibodies such as tendency to precipitate or aggregate. It is incumbent upon Wands to provide reasonable support for the proposed breadth of his claims. I agree with the Commissioner that four exemplars shown to have the desired properties, out of the 143, do not provide adequate support.

Wands argues that the law should not be "harsher" where routine experiments take a long time. However, what Wands is requesting is that the law be less harsh. As illustrated in extensive precedent on the question of how much experimentation is "undue", each case must be determined on its own facts. *See, e.g., W.L. Gore & Assocs., Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1557, 220 USPQ 303, 316 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984); *In re Angstadt*, 537 F.2d 498, 504, 190 USPQ 214, 218 (CCPA 1976); *In re Cook*, 439 F.2d 730, 734-35, 169 USPQ 298, 302-03 (CCPA 1971).

The various criteria to be considered in determining whether undue experimentation is required are discussed in, for example, *Fields v. Conover*, 443 F.2d 1386, 170 USPQ 276 (CCPA 1971); *In re Rainer*, 347 F.2d 574, 146 USPQ 218 (CCPA 1965); *Ex parte Forman*, 230 USPQ at 547. Wands must provide sufficient data or authority to show that his results are reasonably predictable within the scope of the claimed generic invention, based on experiment and/or scientific theory. In my view he has not met this burden.

Patent and Trademark Office
Trademark Trial and Appeal Board

In re Johanna Farms Inc.

Serial No. 542,343

Decided June 30, 1988

JUDICIAL PRACTICE AND PROCEDURE

1. Procedure — Prior adjudication — In general (§410.1501)

Trademark Trial and Appeal Board's prior decision upholding examiner's refusal to register proposed mark "La Yogurt" does not preclude registration of mark pursuant to subsequent application, since applicant, by presenting survey evidence and consumer letters regarding issue of how purchasers perceive proposed mark, has demonstrated that instant factual situation is different from situation presented in prior proceeding.

TRADEMARKS AND UNFAIR TRADE PRACTICES

2. Types of marks — Non-descriptive — Particular marks (§327.0505)

Term "La Yogurt," with "yogurt" disclaimed, is registrable, since word "yogurt" is common English generic term rather than corruption or misspelling of French word for yogurt, since examining attorney failed to meet burden of showing clear evidence of generic use of mark as whole, and since evidence of record, including survey and consumer letters to applicant, demonstrates that primary significance of "La Yogurt" to majority of relevant public is that of brand name rather than generic term.

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TREATMENT OF RHEUMATOID ARTHRITIS WITH CHIMERIC MONOCLONAL ANTIBODIES TO TUMOR NECROSIS FACTOR α

MICHAEL J. ELLIOTT, RAVINDER N. MAINI, MARC FELDMANN, ALICE LONG-FOX, PETER CHARLES, PETER KATSIKIS, FIONULA M. BRENNAN, JEAN WALKER, HANNY BIJL, JOHN GHRAYEB, and JAMES N. WOODY

Objective. **To evaluate the safety and efficacy of a chimeric monoclonal antibody to tumor necrosis factor α (TNFα) in the treatment of patients with rheumatoid arthritis (RA).**

Methods. **Twenty patients with active RA were treated with 20 mg/kg of anti-TNFα in an open phase I/II trial lasting 8 weeks.**

Results. **The treatment was well tolerated, with no serious adverse events. Significant improvements were seen in the Ritchie Articular Index, which fell from a median of 28 at study entry to a median of 6 by week 6 ($P < 0.001$), the swollen joint count, which fell from 18 to 5 ($P < 0.001$) over the same period, and in the other major clinical assessments. Serum C-reactive protein levels fell from a median of 39.5 mg/liter at study entry to 8 mg/liter at week 6 ($P < 0.001$), and significant decreases were also seen in serum amyloid A and interleukin-6 levels.**

Conclusion. **Treatment with anti-TNFα was safe and well tolerated and resulted in significant clinical and laboratory improvements. These preliminary results support the hypothesis that TNFα is an important regulator in RA, and suggest that it may be a useful new therapeutic target in this disease.**

Despite optimal use of current antirheumatic therapy, the outcome for many patients with rheumatoid arthritis (RA) consists of pain, disability, and premature death (1–3). As a response to the need for more effective and less toxic treatment, and to an increase in our understanding of the pathogenic mechanisms in RA, several groups have used monoclonal antibodies as therapeutic agents in this disease (4–10). Such immunotherapy has been, in most cases, targeted specifically to the T cell, a strategy based on evidence that T cells are involved in the initiation and maintenance of RA (11).

Here, we outline an alternative immunotherapeutic strategy, which involves the use of monoclonal antibodies with specificity for a cytokine, tumor necrosis factor α (TNFα). This approach is based on a body of knowledge regarding the role of cytokines in general, and of TNFα in particular, in the inflammatory process in RA. The first clearly documented study demonstrated the presence of interleukin-1 (IL-1) in RA synovial fluid (12). Subsequently, we and others have reported the presence and local synthesis in

From the Clinical Immunology and Sunley Divisions, Kennedy Institute of Rheumatology, and the Academic Department of Rheumatology, Charing Cross and Westminster Medical School, London, United Kingdom; and Centocor Inc., Malvern, Pennsylvania.

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Michael J. Elliott, MB, BS, PhD, FRACP: Clinical Immunology Division, Kennedy Institute of Rheumatology; Ravinder N. Maini, MB, BChir, FRCP: Clinical Immunology Division, Kennedy Institute of Rheumatology; Marc Feldmann, MB, BS, PhD, FRCPath: Sunley Division, Kennedy Institute of Rheumatology; Alice Long-Fox, RGN: Clinical Immunology Division, Kennedy Institute of Rheumatology; Peter Charles, FIMLS: Department of Rheumatology, Charing Cross and Westminster Medical School; Peter Katsikis, MD, PhD: Sunley Division, Kennedy Institute of Rheumatology; Fionula M. Brennan, PhD: Sunley Division, Kennedy Institute of Rheumatology; Jean Walker, RGN, RM: Clinical Immunology Division, Kennedy Institute of Rheumatology; Hanny Bijl, MD: Centocor, Inc; John Ghrayeb, PhD: Centocor Inc; James N. Woody, MD, PhD: Centocor, Inc.

Address reprint requests to Michael J. Elliott, PhD, Kennedy Institute of Rheumatology, 6 Bute Gardens, London W6 7DW, United Kingdom.

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Table 1. Demographic features of 20 patients with refractory rheumatoid arthritis

Patient	Age/sex	Disease duration (years)	Previous DMARDs*	Concomitant therapy†
1	48/F	7	SSZ, DP, GST, AUR, MTX, AZA, HCQ	Pred. 5 mg
2	63/F	7	SSZ, GST, DP	Para. 1–2 gm
3	59/M	3	AUR, HCQ, GST, MTX, SSZ	Pred. 10 mg, Indo. 225 mg
4	56/M	10	GST, DP, AZA, SSZ	Pred. 12.5 mg, Ibu. 2 gm, Para. 1–2 gm
5	28/F	3	GST, SSZ, DP, AZA	Pred. 8 mg, Para. 1–2 gm, Code. 16 mg
6	40/M	3	SSZ, HCQ, AUR	Nap. 1 gm
7	54/F	7	DP, GST, SSZ, AZA, MTX	Para. 1–2 gm, Code. 16–32 mg
8	23/F	11	HCQ, GST, SSZ, MTX, AZA	Pred. 7.5 mg, Dicl. 100 mg, Para. 1–2 gm, Dext. 100–200 mg
9	51/F	15	GST, HCQ, DP, MTX	Pred. 7.5 mg, Dicl. 125 mg, Para. 1–3 gm
10	47/F	12	SSZ, CYC, MTX	Ben. 4 gm
11	34/F	10	DP, SSZ, MTX	Pred. 10 mg, Para. 1.5 gm, Code. 30–90 mg
12	57/F	12	GST, MTX, DP, AUR	Asp. 1.2 gm
13	51/F	7	SSZ, AZA	Para. 1–4 gm
14	72/M	11	GST, DP, AZA, MTX	Pred. 5 mg, Para. 1–4 gm, Code. 16–64 mg
15	51/F	17	HCQ, DP, SSZ, MTX	Asp. 0.3 gm
16	62/F	16	GST, DP, SSZ, MTX, AZA	Para. 1–4 gm, Code. 16–64 mg
17	56/F	11	SSZ, DP, GST, MTX, HCQ, AZA	Pred. 7.5 mg, Eto. 600 mg, Para. 1–2 gm, Dext. 100–200 mg
18	48/F	14	GST, MTX, DP, SSZ, AUR, AZA	Pred. 7.5 mg, Indo. 100 mg, Para. 1–3 gm
19	42/F	3	SSZ, MTX	Fen. 450 mg, Ben. 6 gm, Code. 30 mg
20	47/M	20	GST, DP, SSZ, AZA	Pred. 10 mg, Para. 1–3 gm

* Disease-modifying antirheumatic drugs (DMARDs) were SSZ = sulfasalazine; DP = D-penicillamine; GST = gold sodium thiomalate; AUR = auranofin; MTX = methotrexate; AZA = azathioprine; HCQ = hydroxychloroquine; CYC = cyclophosphamide.
† Daily doses are shown. Pred. = prednisolone; Para. = paracetamol; Indo. = indomethacin; Ibu. = ibuprofen; Code. = codeine phosphate; Nap. = naprosyn; Dicl. = diclofenac; Dext. = dextropropoxyphene; Ben. = benorylate; Asp. = aspirin; Eto. = etodolac; Fen. = fenbufen.

rheumatoid synovial membrane of many cytokines, including IL-1 (13), TNFα (13,14), IL-6 (15), granulocyte-macrophage colony-stimulating factor (GM-CSF; 16), IL-8 (17), and transforming growth factor β (TGFβ) (18,19).

We have investigated the relationships between these cytokines in RA, using a synovial culture system in which dissociated rheumatoid synovial cells are allowed to spontaneously re-aggregate in vivo. Even in the absence of extrinsic stimulation, such cells express high levels of cytokines and HLA class II molecules (20). Using this system, we showed that production of bioactive IL-1 was abrogated by neutralizing antibodies to TNFα, but not by antibodies to TNFβ or by normal rabbit IgG (21). This occurred in rheumatoid, but not osteoarthritic, cultures and suggested to us that TNFα was of particular importance as a regulatory cytokine. Subsequent analysis reinforced this concept, with the demonstration that another proinflammatory cytokine, GM-CSF, was regulated in the synovial membrane by TNFα (22) and that TNFα receptor expression, necessary for transmitting TNFα signals, was up-regulated in rheumatoid synovium (23,24).

Two recent mouse studies provide further insight into the importance of TNFα in arthritis. Keffer et al (25) described a mouse transgenic for the human TNFα gene, which expressed high levels of human TNFα in vivo and which reproducibly developed arthritis beginning at 4 weeks of age. The disease in these animals could be prevented by administration of monoclonal antibodies to human TNFα. In separate experiments in our own laboratory, we showed that in the type II collagen arthritis model in the DBA/1 mouse, the hamster anti-murine TNF monoclonal antibody TN3.19.2 significantly ameliorated the inflammation and tissue destruction when administered before or after the onset of disease (26).

Based on these considerations, it was of interest to determine the effect of therapy with a chimeric (human IgG1, murine Fv) monoclonal antibody to human TNFα in patients with rheumatoid arthritis. We report here that anti-TNFα therapy was safe and well tolerated, and induced marked improvements in both clinical and laboratory disease measures. These findings are consistent with our postulate concerning the critical role of TNFα in the pathogenesis of RA (27,28), and suggest that TNFα may be a useful therapeutic target in this disease.

Table 2. Changes in clinical assessments following treatment of rheumatoid arthritis patients with cA2*

Week of trial	Morning stiffness, minutes	Pain score, 0–10 cm	Ritchie index, 0–69	Swollen joint count, 0–28	Grip strength, 0–300 mm Hg		IDA, 1–4	Patient's assessment, no. grades improved, 0–3
					Left hand	Right hand		
Screen	135, 0–600	7.4, 4–9.7	23, 4–51	16, 4–28	84, 45–300	96, 57–300	3, 2.3–3.3	NA
0	180, 20–600	7.1, 2.7–9.7	28, 4–52	18, 3–27	77, 52–295	92, 50–293	3, 2–3.5	NA
1	20, 0–180 (<0.001†)	2.6, 0.6–7.8 (<0.001†)	13, 2–28 (<0.001; <0.002†)	13.5, 1–25 (>0.05)	122, 66–300 (>0.05)	133, 57–300 (>0.05)	2, 1.5–3.3 (<0.001†)	1, 1–3
2	15, 0–150 (<0.001†)	3.0, 0.3–6.4 (<0.001†)	13, 1–28 (<0.001†)	11.5, 1–22 (<0.003; <0.02†)	139, 75–300 (<0.03; >0.05†)	143, 59–300 (>0.05)	2, 1.5–3.2 (<0.001†)	1.5, 1–3
3	5, 0–150 (<0.001†)	2.2, 0.2–7.4 (<0.001†)	8, 0–22 (<0.001†)	6, 1–19 (<0.001; <0.002†)	113, 51–300 (>0.05)	142, 65–300 (>0.05)	2, 1.2–3.2 (<0.001†)	2, 1–2
4	15, 0–90 (<0.001†)	1.9, 0.1–5.6 (<0.001†)	10, 0–17 (<0.001†)	6, 0–21 (<0.001; <0.002†)	124, 79–300 (<0.02; >0.05†)	148, 64–300 (<0.03; >0.05†)	1.8, 1.3–2.7 (<0.001†)	2, 1–2
6	5, 0–90 (<0.001†)	1.9, 0.1–6.2 (<0.001†)	6, 0–18 (<0.001†)	5, 1–14 (<0.001†)	119, 68–300 (<0.04; >0.05†)	153, 62–300 (<0.05; >0.05†)	1.7, 1.3–2.8 (<0.001†)	2, 1–2
8	15, 0–60 (<0.001†)	2.1, 0.2–7.7 (<0.001†)	8, 1–28 (<0.001†)	7, 1–18 (<0.001†)	117, 69–300 (<0.03; >0.05†)	167, 53–300 (<0.03; >0.05†)	1.8, 1.5–2.8 (<0.001†)	2, 1–3

* Values are the median, range (*P*) for 20 patients for the initial screen and weeks 0–2, and for 19 patients thereafter. Patient 15 dropped out after week 2 of study. All *P* values versus week 0, by Mann-Whitney test. IDA = Index of Disease Activity; NA = not applicable.
† Adjusted for multiple statistical comparisons.

PATIENTS AND METHODS

Patient selection. Twenty patients were recruited, each of whom fulfilled the American College of Rheumatology (formerly, the American Rheumatism Association) criteria for the diagnosis of RA (29). The clinical characteristics of the patients are shown in Table 1. The study group comprised 15 females and 5 males, with a median age of 51 years (range 23–72), a median disease duration of 10.5 years (range 3–20), and a history of failed therapy with standard disease-modifying antirheumatic drugs (DMARDs) (median number of failed DMARDs 4, range 2–7).

Seventeen patients were seropositive at study entry or had been seropositive at some stage of their disease. All had erosions evident on radiographs of the hands or feet, and 3 had rheumatoid nodules. All patients had active disease at trial entry, as defined by an Index of Disease Activity (IDA) (30) of at least 1.75, together with at least 3 swollen joints, and were classified in anatomic and functional stage II or III (31). The pooled data for each of the clinical and laboratory indices of disease activity at the time of screening for the trial (up to 4 weeks prior to trial entry), and on the day of trial entry itself (week 0), are shown in Tables 2 and 3.

All DMARDs were discontinued at least 1 month prior to trial entry. Patients were allowed to continue taking a nonsteroidal antiinflammatory drug and/or prednisolone (≤12.5 mg/day) during the trial. The dosage of these agents was kept stable for 1 month prior to trial entry and during the course of the trial. No parenteral corticosteroids were allowed during these periods. Simple analgesics were allowed ad libitum.

Patients with other serious medical conditions were excluded from study. Specific exclusions were as follows: serum creatinine >150 μmoles/liter (normal 60–120), hemoglobin (Hgb) <90 gm/liter (normal 120–160 in females, and 135–175 in males), white blood cell (WBC) count <4 × 10^9/liter (normal 4–11 × 10^9/liter), platelet count <100 × 10^9/liter (normal 150–400 × 10^9/liter), and abnormal liver enzyme levels or active pathology noted on chest radiographs.

All patients gave their informed consent for the trial, and approval was granted by the local ethics committee.

Treatment protocol. cA2 is a chimeric human/mouse monoclonal anti-TNFα antibody, consisting of the constant regions of human (Hu)IgG1κ, coupled to the Fv region of a high-affinity neutralizing murine anti-HuTNFα antibody (A2). The antibody was produced by Centocor Inc., by continuous fermentation of a mouse myeloma cell line which had been transfected with cloned DNA coding for cA2, and was purified from culture supernatant by a series of steps involving column chromatography. The chimeric antibody retains specificity for natural and recombinant HuTNFα, and is of high affinity.

The antibody was stored at 4°C in 20-ml vials containing 5 mg of cA2 per milliliter of 0.01*M* phosphate buffered saline in 0.15*M* sodium chloride at a pH of 7.2 and was filtered through a 0.2-μm sterile filter before use. The appropriate amount of cA2 was then diluted to a total volume of 300 ml in sterile saline and administered intravenously via a 0.2-μm in-line filter over a period of 2 hours.

Patients were admitted to the hospital for 8–24 hours for each treatment, and were mobile except during infusions. The trial was of an open, uncontrolled design, with a comparison of 2 treatment schedules. Patients 1–5 and 11–20 received a total of 2 infusions, each consisting of 10 mg/kg of cA2, at entry to the study (week 0) and 14 days later (week 2). Patients 6–10 received a total of 4 infusions of 5 mg/kg at cA2, at entry and on days 4, 8, and 12. The total dose received by the 2 patient groups was therefore the same: 20 mg/kg.

Assessments. *Safety monitoring.* Vital signs were recorded every 15–30 minutes during infusions, and at intervals for up to 24 hours postinfusion. Patients were

Table 3. Changes in laboratory measures following treatment of rheumatoid arthritis patients with cA2*

Week of trial	Hgb, gm/liter	WBC, $\times 10^9$/liter	Platelets, $\times 10^9$/liter	ESR, mm/hour	CRP, mg/liter	SAA, mg/ml	RF, inverse titer
Screen	117, 98–146	7.9, 3.9–15.2	352, 274–631	59, 18–87	42, 9–107	ND	ND
0	113, 97–144	9.0, 4.9–15.7	341, 228–710	55, 15–94	39.5, 5–107	245, 18–1,900	2,560, 160–10,240
1	114, 96–145 (>0.05)	8.5, 3.6–13.6 (>0.05)	351, 223–589 (>0.05)	26, 13–100 (>0.05)	5, 0–50 (<0.001†)	58, 0–330 (<0.001; <0.003†)	ND
2	112, 95–144 (>0.05)	8.2, 4.3–12.7 (>0.05)	296, 158–535 (<0.04; >0.05†)	27, 10–90 (<0.02; >0.05†)	5.5, 0–80 (<0.001; <0.003†)	80, 11–900 (<0.02; <0.04†)	ND
3	110, 89–151 (>0.05)	9.0, 3.7–14.4 (>0.05)	289, 190–546 (<0.03; >0.05†)	27, 12–86 (<0.04; >0.05†)	7, 0–78 (<0.001; <0.002†)	ND	ND
4	112, 91–148 (>0.05)	8.2, 4.7–13.9 (>0.05)	314, 186–565 (>0.05)	23, 10–87 (<0.04; >0.05†)	10, 0–91 (<0.004; <0.02†)	ND	ND
6	116, 91–159 (>0.05)	9.1, 2.9–13.9 (>0.05)	339, 207–589 (>0.05)	23, 12–78 (<0.03; >0.05†)	8, 0–59 (<0.001†)	ND	ND
8	114, 94–153 (>0.05)	7.6, 4.2–13.5 (>0.05)	339, 210–591 (>0.05)	30, 7–73 (>0.05)	6, 0–65 (<0.001†)	ND	480, 40–5,120 (>0.05)

* Values are the median, range (*P*) for 20 patients for the initial screen and weeks 0–2, and for 19 patients thereafter. Patient 15 dropped out after week 2 of study. For rheumatoid factor (RF), only those patients with week 0 titers ≥1:160 in the particle agglutination assay were included (n = 14). All *P* values versus week 0, by Mann-Whitney test. Normal ranges: hemoglobin (Hgb) 120–160 gm/liter in females and 135–175 gm/liter in males; white blood cell (WBC) count 4–11 × 10^9/liter; platelet count 150–400 × 10^9/liter; erythrocyte sedimentation rate (ESR) <15 mm/hour in females and <10 mm/hour in males; C-reactive protein (CRP) <10 mg/liter; serum amyloid A (SAA) <10 mg/ml. ND = not done.

questioned concerning possible adverse events before each infusion and at weeks 1, 2, 3, 4, 6, and 8 of the trial. A complete physical examination was performed at screening and at week 8. In addition, patients were monitored by standard laboratory tests including a complete blood cell count, and levels of C3 and C4 components of complement, IgG, IgM, and IgA, serum electrolytes, creatinine, urea, alkaline phosphatase, aspartate transaminase, and total bilirubin.

Sample times for these tests were between 0800 and 0900 hours (preinfusion) and 1200–1400 hours (24 hours postinfusion). Blood tests subsequent to day 1 were performed in the morning, usually between 0700 and 1200 hours. Urine analysis and culture were also performed at each assessment point.

Response assessment. The patients were assessed for response to cA2 at weeks 1, 2, 3, 4, 6, and 8 of the trial. The assessments were all made between 0700 and 1300 hours by the same observer (AL-F). The following clinical assessments were made: duration of morning stiffness (minutes), pain score (0–10 cm on a visual analog scale), Ritchie Articular Index (maximum score 69) (32), number of swollen joints (28 joint count) (validation described in ref. 33), grip strength (0–300 mm Hg, mean of 3 measurements per hand, by sphygmomanometer cuff), and an assessment of function (the Stanford Health Assessment Questionnaire [HAQ], modified for British patients [34]). In addition, the patients' global assessments of response were recorded using a 5-point scale (worse, no response, fair response, good response, excellent response).

Routine laboratory indicators of disease activity included complete blood cell counts, C-reactive protein (CRP) levels (by rate nephelometry), and the erythrocyte sedimentation rate (ESR; Westergren). Followup assessments were made at monthly intervals after the conclusion of the formal trial period, in order to assess the duration of response.

Analysis of improvement in individual patients was made using two separate indices. First, an IDA was calculated for each time point according to the method of Mallya and Mace (30), with input variables of morning stiffness, pain score, Ritchie Articular Index, grip strength, ESR, and Hgb. The second index calculated was that of Paulus et al (35), which uses input variables of morning stiffness, ESR, joint pain/tenderness, joint swelling, and patient's and physician's global assessments of disease severity.

To calculate the presence (or otherwise) of a response according to this index, two approximations were made to accommodate our data. The swollen joint count used by us (nongraded total of swollen joints of 28 joints assessed), which has been validated (33), was used in place of the more extensive graded count described by Paulus et al, and the patient's and physician's global assessments of *response* recorded by us were approximated to the global assessments of *disease activity* used by Paulus et al (35). In addition to determining response according to these published indices, we selected 6 disease activity assessments of interest (morning stiffness, pain score, Ritchie Articular Index, swollen joint count, ESR, and CRP) and calculated their mean percentage improvement. We have used this value to give an indication of the degree of improvement seen in responding patients.

Immunologic investigations. Rheumatoid factors were measured using the rheumatoid arthritis particle agglutination assay (RAPA) (FujiBerio Inc, Tokyo, Japan), in which titers of 1:160 or greater were considered significant. Rheumatoid factor isotypes were measured by enzyme-linked immunosorbent assay (ELISA) (Cambridge Life Sciences, Ely, UK). Addition of cA2, at concentrations of up to 200 μg/ml, to these assay systems did not alter the assay results (data not shown).

Antinuclear antibodies were detected by immunoflu-

orescence on HEp-2 cells (Biodiagnostics, Upton, UK), and antibodies to extractable nuclear antigens were measured by counterimmunoelectrophoresis with polyantigen extract (Biodiagnostics). Sera positive by immunofluorescence were also screened for antibodies to DNA by the Farr assay (Kodak Diagnostics, Amersham, UK). Anticardiolipin antibodies were measured by ELISA (Shield Diagnostics, Dundee, Scotland). Serum amyloid A (SAA) was measured by sandwich ELISA (Biosource International, Camarillo, CA). Antiglobulin responses to the infused chimeric antibody were measured by an in-house ELISA, using cA2 as a capture reagent.

Cytokine assays. Bioactive TNF was measured in sera using the WEHI 164 clone 13 cytotoxicity assay (36). Total IL-6 was measured in sera using a commercial immunoassay (Medgenix Diagnostics, Brussels, Belgium) and using a sandwich ELISA developed in-house, with monoclonal antibodies provided by Dr. F. di Padova (Basel, Switzerland). Microtiter plates were coated with monoclonal antibody LNI 314-14 at a concentration of 3 μg/ml for 18 hours at 4°C, and blocked with 3% bovine serum albumin in 0.1*M* phosphate buffered saline, pH 7.2. Undiluted sera or standards (recombinant HuIL-6, 0–8.1 μg/ml) were added to the wells in duplicate and incubated for 18 hours at 4°C. Bound IL-6 was detected by incubation with monoclonal antibody LNI 110-14 for 90 minutes at 37°C, followed by biotin-labeled goat anti-murine IgG2b for 90 minutes at 37°C (Southern Biotechnology, Birmingham, AL). The assay was developed using streptavidin–alkaline phosphatase (Southern Biotechnology) and *p*-nitrophenyl phosphate as a substrate, and the optical density read at 405 nm.

Statistical analysis. Data for week 0 versus subsequent time points were compared for each assessment using the Mann-Whitney test. For comparison of rheumatoid factor titers (by RAPA), the data were expressed as dilutions before applying the test.

This was an exploratory study, in which prejudgments about the optimal times for assessment were not possible. Although it has not been common practice to adjust for multiple statistical comparisons in such studies (4–10), a conservative statistical approach would require adjustment of *P* values to take into account analysis at several time points. The *P* values have therefore been presented in two forms: unadjusted, and after making allowance for analysis at multiple time points by use of the Bonferroni adjustment. Where *P* values remained <0.001 after adjustment, a single value only is given. A *P* value of <0.05 is considered significant.

RESULTS

Safety of cA2. The administration of cA2 was exceptionally well tolerated, with no headache, fever, hemodynamic disturbance, allergy, or other acute manifestation. No serious adverse events were recorded during the 8-week trial. Two minor infective episodes were recorded, each "possibly related" to cA2: patient 15 presented at week 2 with clinical features of bronchitis. Sputum culture grew only normal commensals. She had a history of smoking and of a similar illness 3 years previously. The illness responded promptly to treatment with amoxicillin, but her second cA2 infusion was withheld and the data for this patient are therefore not analyzed beyond week 2. Patient 18 showed significant bacteriuria on routine culture at week 6 (>10^5/ml; lactose-fermenting coliform), but was asymptomatic. This condition also responded promptly to amoxicillin.

Routine analysis of blood samples showed no consistent adverse changes in hematologic parameters, renal function, liver function, or levels of C3, C4, or immunoglobulins during the 8 weeks of the trial. Four minor, isolated, and potentially adverse laboratory disturbances were recorded. Patient 2 experienced a transient rise in blood urea levels, from 5.7 mmoles/liter to 9.2 mmoles/liter (normal 2.5–7), with no change in serum creatinine. This change was associated with the temporary use of a diuretic, which had been prescribed for a non-rheumatologic disorder. The value normalized within 1 week and was classified as "probably not related" to cA2.

Patient 6 experienced a transient fall in the peripheral blood lymphocyte count, from 1.6 × 10^9/liter to 0.8 × 10^9/liter (normal 1.0–4.8). This abnormality was not seen at the next sample point (2 weeks later), was not associated with any clinical manifestations, and was classified as "possibly related" to cA2. Patients 10 and 18 developed elevated titers of anti-DNA antibodies at weeks 6 and 8 of the trial. Elevated anticardiolipin antibodies were also detected in patient 10. Both patients had a preexisting positive antinuclear antibody titer, and patient 10 had a history of borderline lymphocytopenia and high serum IgM. There were no clinical features of systemic lupus erythematosus, and the laboratory changes were judged "probably related" to cA2.

Efficacy of cA2. The pattern of response for each of the clinical assessments of disease activity and the derived IDA are shown in Table 2. All clinical assessments showed improvement following treatment with cA2, with maximal responses from week 3. Duration of morning stiffness decreased from a median of 180 minutes at study entry (week 0) to 5 minutes at week 6 ($P < 0.001$ by Mann-Whitney test, adjusted), representing a 97% improvement. The pain score decreased from 7.1 to 1.9 over the same period ($P < 0.001$, adjusted), representing an improvement of 73%. Similarly, the Ritchie Articular Index improved from 28 to 6 at week 6 ($P < 0.001$, adjusted; 79% improvement), and the swollen joint count decreased from 18

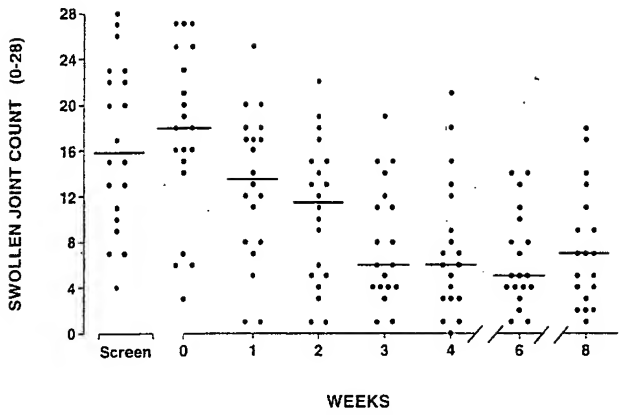

Figure 1. Swollen joint counts (maximum 28), as recorded by a single observer, in 20 patients with rheumatoid arthritis treated with cA2. The screening time point was within 4 weeks of entry to the study (week 0); data from patient 15 were not included after week 2 (dropout). Significance of the changes, relative to week 0, were determined by Mann-Whitney test (adjusted): $P > 0.05$ at week 1, $P < 0.02$ at week 2, $P < 0.002$ at weeks 3 and 4, and $P < 0.001$ at weeks 6 and 8. Bars show median values.

to 5 ($P < 0.001$, adjusted; 72% improvement). The individual swollen joint counts for all time points are shown in Figure 1.

Grip strength also improved; the median grip strength rose from 77 mm Hg (left) and 92 mm Hg (right) at week 0 to 119 (left) and 153 (right) at week 6 ($P < 0.04$ and $P < 0.05$, left and right hands, respectively; $P > 0.05$ both hands, adjusted for multiple comparisons). The IDA has a range of 1 (normal) to 4 (severe disease activity). The IDA showed a decrease from a median of 3 at study entry to 1.7 at week 6 ($P < 0.001$, adjusted). Patients were asked to grade their responses to cA2 using a 5-point scale. No patient recorded a response of "worse" or "no change" at any point in the trial. "Fair," "good," and "excellent" responses were classified as improvements of 1, 2, and 3 grades, respectively. At week 6, there was a median of 2 grades of improvement (Table 2).

We also measured changes in the patients' functional capacity, using the HAQ, as modified for British patients (range 0–3). The median (range) HAQ score improved from 2 (0.9–3) at study entry to 1.1 (0–2.6) by week 6 ($P < 0.001$ and $P < 0.002$ adjusted).

The changes in the laboratory values which reflect disease activity are shown in Table 3. The most rapid and impressive changes were seen in serum CRP levels, which fell from a median of 39.5 mg/liter at week 0 (normal <10) to 8 mg/liter by week 6 of the trial ($P < 0.001$, adjusted), representing an improvement of 80%. Of the 19 patients with elevated CRP at study entry, 17 showed decreases to the normal range at some point during the trial. The improvement in CRP was maintained in most patients over the assessment period (Table 3 and Figure 2); the exceptions with high values at 4 and 6 weeks tended to be those with the highest starting values (data not shown).

The ESR also showed improvement, with a fall from 55 mm/hour at study entry (normal <10 in males and <15 in females) to 23 mm/hour at week 6 ($P < 0.03$ and $P > 0.05$ adjusted; 58% improvement). SAA levels were elevated in all patients at trial entry, and fell from a median of 245 mg/ml (normal <10) to 58 mg/ml at week 1 ($P < 0.003$ adjusted; 76% improvement) and to 80 mg/ml at week 2 ($P < 0.04$, adjusted). No significant changes were seen in Hgb level, WBC count, or platelet count at week 6, although the platelet count did improve at weeks 2 and 3 compared with trial entry (Table 3).

The response data were also analyzed for each patient individually (not shown). The majority of patients had their best overall responses at week 6, at which time 13 assessed their responses as "good" while 6 assessed their responses as "fair." Eighteen of the 19 patients who completed the treatment schedule achieved an improvement in the IDA of 0.5 or greater at week 6, and 10 achieved an improvement of 1.0 or greater. All patients achieved a response at week 6

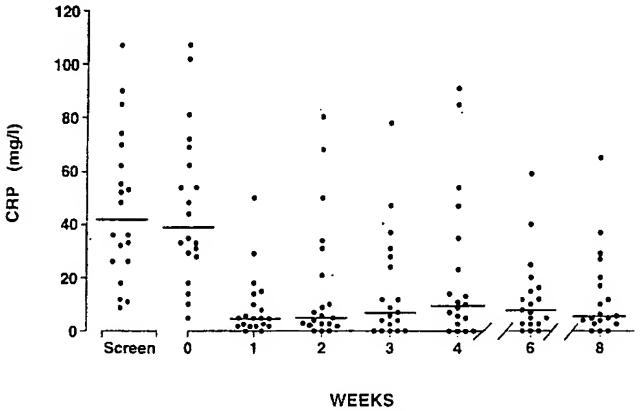

Figure 2. Serum C-reactive protein (CRP) levels (normal 0–10 mg/liter), as measured by nephelometry, in 20 patients with rheumatoid arthritis treated with cA2. The screening time point was within 4 weeks of entry to the study (week 0); data from patient 15 were not included after week 2 (dropout). Significance of the changes, relative to week 0, were determined by Mann-Whitney test (adjusted): $P < 0.001$ at week 1, $P < 0.003$ at week 2, $P < 0.002$ at week 3, $P < 0.02$ at week 4, and $P < 0.001$ at weeks 6 and 8. Bars show median values.

according to the index described by Paulus et al (35). At week 6, all patients showed a mean improvement of 30% or greater in the 6 selected measures of disease activity (see Patients and Methods), with 18 of the 19 patients showing a mean improvement of 50% or greater (data not shown).

Although the study was primarily designed to assess the short-term effects of cA2 treatment, followup clinical and laboratory data are available for those patients followed for sufficient time (n = 12). The duration of response in these patients, defined as the duration of a 30% (or greater) mean improvement in the 6 selected disease activity measures, was variable, ranging from 8 weeks to 25 weeks (median 14) (data not shown).

Comparison of the clinical and laboratory data for patients treated with 2 infusions of cA2 (each at 10 mg/kg) versus those treated with 4 infusions (each at 5 mg/kg) showed no significant differences in the rapidity or extent of response (data not shown).

Immunologic investigations and cytokines. Measurement of rheumatoid factor by RAPA showed 14 patients with significant titers (≥1:160) at trial entry. Of these, 6 patients showed a decrease of at least 2 titers on treatment with cA2, while the remaining patients showed a change of 1 titer or less. No patient showed a significant increase in rheumatoid factor titer during the trial (data not shown). The median titer in the 11 patients decreased from 1:2,560 at entry to 1:480 by week 8 ($P > 0.05$) (Table 3). Specific rheumatoid factor isotypes were measured by ELISA, and showed decreases in the 6 patients whose RAPA had declined significantly, as well as in some other patients (data not shown). Median values for the 3 isotypes in the 14 patients seropositive at trial entry were 119, 102, and 62 IU/ml (IgM, IgG, and IgA isotypes, respectively) and at week 8 were 81, 64, and 46 IU/ml ($P > 0.05$).

We tested sera from patients 1–9 for the presence of bioactive TNF, using the WEHI 164 clone 13 cytotoxicity assay (36). In 8 patients, serum samples spanning the entire trial period were tested; while for patient 9, only 3 samples (1 pretrial, 1 intermediate, and the last available sample) were tested. The levels of bioactive TNF were below the limit of sensitivity of the assay in the presence of human serum (1 pg/ml) (data not shown).

Since production of CRP and SAA are thought to be regulated in large part by IL-6, we also measured serum levels of this cytokine, using 2 different assays which measure total IL-6. In the Medgenix assay, IL-6 was significantly elevated in 17 of the 20 patients at study entry. In this group, levels fell from 60 pg/ml (range 18–500) to 40 pg/ml (range 0–230) at week 1 ($P > 0.05$) and to 32 pg/ml (range 0–210) at week 2 ($P < 0.005$ and $P < 0.01$, adjusted). These results were supported by measurement of serum IL-6 in the first 16 patients in a separate ELISA developed in-house. IL-6 was detectable in 11 of these samples, with median (range) levels falling from 210 pg/ml (25–900) at entry to 32 pg/ml (0–1,700) at week 1 ($P < 0.02$ and $P < 0.04$, adjusted) and to 44 pg/ml (0–240) at week 2 ($P < 0.02$ and $P < 0.03$, adjusted).

We tested sera from patients 1–10 for the presence of antiglobulin responses to the infused chimeric antibody, but none were detected (data not shown). In many patients, however, cA2 was still detectable in serum samples taken at week 8 (data not shown) and this may have interfered with the ELISA.

DISCUSSION

This is the first report describing the administration of anti-TNFα antibodies for treatment of human autoimmune disease. Many cytokines are produced in rheumatoid synovium, but we chose to specifically target TNFα because of mounting evidence that it was a major molecular regulator in RA (21,22,26–28). The study results presented here support that view and allow 3 important conclusions to be drawn.

First, treatment with cA2 was safe and the infusion procedure was well tolerated. Although fever, headache, chills, and hemodynamic disturbance have all been reported following treatment with anti-CD4 or anti-CDw52 in RA (6,10), such features were absent in our patients. Also notable was the absence of any allergic event despite repeated treatment with the chimeric antibody, although the interval between initial and repeat infusions may have been too short to allow maximal expression of any antiglobulin response. The continuing presence of circulating cA2 at the conclusion of the trial may have precluded detection of antiglobulin responses, but also indicated that any such responses were likely to be of low titer and/or affinity. Although we recorded 2 episodes of infection among the study group, these were minor and the clinical courses were unremarkable. TNFα has been implicated in the control of *Listeria* and other infections in mice (37), but our limited experience does not suggest an increased risk of infection after TNFα blockade in humans.

The second conclusion concerns the clinical

efficacy of cA2. The patients we treated had longstanding, erosive, and for the most part, seropositive disease, and therapy with several standard DMARDs had failed. Despite this, the major clinical assessments of disease activity and outcome (morning stiffness, pain score, Ritchie articular index, swollen joint count, and HAQ score) showed statistically significant improvement, even after adjustment for multiple comparisons. All patients graded their response as at least "fair," with the majority grading it as "good." In addition, all achieved a response according to the criteria of Paulus et al and showed a mean improvement of at least 30% in 6 selected disease activity measures. The design of the trial does not allow these results to be attributed to the action of cA2 alone. However, the extent of the clinical improvements, their consistency throughout the study group, and the parallel changes in laboratory indices of disease activity (see below) are encouraging.

The improvements in clinical assessments following treatment with cA2 appear to be at least as good as those reported following treatment of similar patients with antileukocyte antibodies (6,10), although firm conclusions concerning each of these agents will require controlled, blinded studies. The two therapeutic approaches may already be distinguished, however, by their effects on the acute-phase response, since in several studies of antileukocyte antibodies, no consistent improvements in CRP or ESR were seen (4–6,8,10). In contrast, treatment with cA2 resulted in significant decreases in serum CRP and SAA values, with normalization of values in many patients. The changes were rapid and marked, and in the case of CRP, sustained for the duration of the study (Table 3). The decreases in ESR were less marked, achieving statistical significance only when unadjusted for the number of comparisons (Table 3).

These results are consistent with current concepts that implicate TNFα in the regulation of hepatic acute-phase protein synthesis, either directly, or by control of other, secondary, cytokines such as IL-6 (38,39). To investigate the mechanism of control of the acute-phase response in our patients, we measured serum TNFα and IL-6 before and after cA2 treatment. Bioactive TNFα was not detectable in sera obtained at baseline or subsequently. In view of previous reports of variability between different immunoassays in the measurement of cytokines in biologic fluids (40), we used 2 different assays for IL-6, and both demonstrated significant decreases in serum IL-6 levels by week 2. These findings support the other objective laboratory changes induced by cA2, and provide in vivo evidence that TNFα may be a regulatory cytokine for IL-6 in this disease. Among the other laboratory tests performed, levels of rheumatoid factors fell significantly in 6 patients.

The mechanism of action of cA2 leading to the clinical responses outlined above was not established in this study. Neutralization of TNFα may have a number of beneficial consequences, including a reduction in the local release of cytokines such as IL-6 and other inflammatory mediators, and modulation of synovial endothelial/leukocyte interactions. cA2 may also bind directly to synovial inflammatory cells expressing membrane TNFα, with subsequent in situ cell lysis. Further studies should establish which actions of cA2 may be clinically important.

The results obtained in this small series have important implications, both scientifically and clinically. At the scientific level, the ability of the neutralizing antibody, cA2, to reduce acute-phase protein synthesis, reduce the production of other cytokines such as IL-6, and significantly improve the clinical state demonstrates that it is possible to interfere with the cytokine network in a useful manner without untoward effects. Due to the many functions and overlapping effects of cytokines such as IL-1 and TNFα, and the fact that cytokines induce the production of other cytokines and of themselves, there had been some pessimism as to whether targeting a single cytokine in vivo would have any beneficial effect (41,42). This view is clearly refuted. On the clinical side, the results of short-term treatment with cA2 are encouraging, and suggest that TNFα may be a useful new therapeutic target in RA.

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Inhibition of immunoreactive tumor necrosis factor-alpha by a chimeric antibody in patients infected with human immunodeficiency virus type 1.

J Infect Dis. 1996 Jul;174(1):63-8. Unique Identifier : AIDSLINE MED/96261994

Walker RE; Spooner KM; Kelly G; McCloskey RV; Woody JN; Falloon J; Baseler M; Piscitelli SC; Davey RT Jr; Polis MA; Kovacs JA; Masur H; Lane HC; National Institute of Allergy and Infectious Diseases, Critical; Care Medicine Department, National Institutes of Health,; Bethesda, MD 20892, USA.

Abstract: Tumor necrosis factor-alpha (TNF-alpha), a proinflammatory cytokine known to stimulate human immunodeficiency virus type 1 (HIV-1) replication, has been implicated in the pathogenesis of HIV-1 infection. Inhibition of TNF-alpha by a chimeric humanized monoclonal antibody, cA2, was investigated in 6 HIV-1-infected patients with CD4 cell counts < 200/mm3. Two consecutive infusions of 10 mg/kg 14 days apart were well tolerated, and a prolonged serum half-life for cA2 (mean, 257 +/- 70 h) was demonstrated. Serum immunoreactive TNF-alpha concentrations fell from a mean prestudy value of 6.4 pg/mL (range, 4.2-7.9) to 1.1 pg/mL (range, 0.5-2.2) 24 h after the first infusion and returned to baseline within 7-14 days. A similar response was seen after the second infusion. No consistent changes in CD4 cell counts or plasma HIV RNA levels were observed over 42 days. Future studies evaluating the therapeutic utility of long-term TNF-alpha suppression using anti-TNF-alpha antibodies are feasible and warranted.

Keywords: Acquired Immunodeficiency Syndrome/BLOOD/*IMMUNOLOGY Adult Animal Antibodies/*THERAPEUTIC USE Antibodies, Monoclonal/*THERAPEUTIC USE Chimeric Proteins/PHARMACOKINETICS/*THERAPEUTIC USE Female Human *HIV-1 Male Mice Recombinant Proteins/PHARMACOKINETICS/THERAPEUTIC USE Tumor Necrosis Factor/*ANTAGONISTS & INHIB/IMMUNOLOGY JOURNAL ARTICLE
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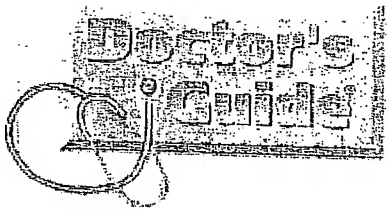

Medical & Other News

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Title: **New Monoclonal Antibody Effective Treatment For Crohn's Disease Therapy**
URL: http://www.pslgroup.com/dg/2802E.htm
Doctor's Guide
May 13, 1997

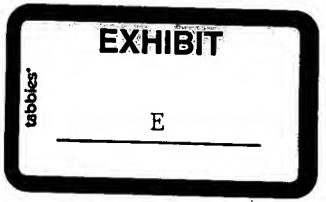
WASHINGTON, and MALVERN, Pa., May 13, 1997 -- Statistically significant results were released yesterday from two controlled clinical studies testing cA2(TM) (infliximab), a monoclonal antibody, in the treatment of Crohn's disease, a chronic disorder characterized by inflammation of the gastrointestinal tract. Data from both trials show that treatment with cA2 can have a beneficial effect on both the severity and number of symptoms associated with Crohn's disease.

"This kind of clinical response in Crohn's disease is unprecedented," said Stephan Targan, M.D., principal investigator and Director of the Inflammatory Bowel Disease Center at Cedars-Sinai Medical Center in Los Angeles, "and provides compelling evidence of the potential of cA2 in the treatment of Crohn's disease."

The results of these trials, which were conducted in 18 centers in North America and Europe, were announced today during Digestive Disease Week in Washington, DC. Digestive Disease Week is sponsored by the American Association for the Study of Liver Diseases, the American Gastroenterological Association, the American Society for Gastrointestinal Endoscopy and The Society for Surgery of the Alimentary Tract.

Last year, during Digestive Disease Week, Centocor released data showing a statistically significant improvement in disease activity following a single infusion of cA2. In the initial study, 65 percent of patients treated with cA2 achieved a clinical response and 33 percent of patients went into remission within four weeks of the start of treatment.

In the extension phase of this study, known as T16, which is being reported today, additional cA2 treatments were demonstrated to maintain Crohn's disease patients in clinical remission as measured by the CDAI, the Crohn's disease activity index.



In the initial phase of the T16 trial, the median CDAI of treated patients dropped from 312 to 125 eight weeks after a single cA2 infusion. Following four additional infusions, given eight weeks apart in the most recent phase of the T16 trial, cA2 maintained the CDAI reduction, with median CDAI eight weeks following the final treatment at 117 (CDAI<150 constitutes disease remission).

Data from the second trial, named T20, indicate that cA2 may be a valuable treatment for enterocutaneous fistulae, a painful, debilitating complication of Crohn's disease in which extensions occur between the bowel and the skin, mostly in the perianal area, causing drainage of mucous and/or fecal material. In this trial, approximately two-thirds of participants experienced closure of at least 50 percent of their fistulae.

In both clinical trials, onset of cA2 clinical benefit was rapid with the vast majority of responders achieving response within two weeks. In addition, cA2 was generally well tolerated in these two trials. "We have been following these studies with great interest," said Richard P. MacDermott, M. D., Immediate Past Chairperson, National Scientific Advisory Committee, Crohn's & Colitis Foundation of America (CCFA). "The results are very encouraging. It is possible that an important new therapy for Crohn's disease may be on the horizon."

In the T16 study, 73 patients who showed a clinical response eight weeks after their initial infusion of cA2 were re-randomized at week 12 to further treatment with cA2 or placebo, and infused every eight weeks for a total of four additional infusions. Those patients re-randomized to cA2 continued to experience an improvement in symptoms from baseline assessment and the percentage of patients achieving clinical remission was maintained at approximately 60 percent during the re-treatment period.

Those patients who responded to their initial infusion of cA2 and then received placebo in the re-treatment phase of the study, experienced a gradual decline in clinical effect over time. However, 19 percent of the placebo group were still in remission 48 weeks after their initial cA2 infusion.

The second study, T20, was conducted with 94 patients with draining enterocutaneous fistulae. Following a series of three cA2 infusions given two and four weeks apart, two-thirds of patients experienced closure of at least 50 percent of their fistulas (P=0.002). These patients had previously failed to respond adequately to treatment with combinations of corticosteroids, methotrexate, 6-MP/azathioprine, aminosalicylates, or antibiotics. These underlying therapies were given in conjunction with the cA2 infusions in this study. "cA2 is the first drug to ever demonstrate statistical significance in a controlled trial to close fistulas," according to Daniel Present, M.D., principal investigator and Clinical Professor of Medicine at Mount Sinai.

cA2, a monoclonal antibody, is the first of a revolutionary class of agents being studied for Crohn's disease. It is a well-tolerated, highly selective treatment that blocks activity of a key inflammatory mediator called tumor necrosis factor or TNF. cA2 is also being studied for treatment of rheumatoid arthritis.

Centocor is a biotechnology company whose mission is to develop and commercialize novel therapeutic and diagnostic products and services that solve critical needs in human health care. The company concentrates on research and development, manufacturing and market development, with a primary technology focus on monoclonal antibodies and DNA-based products.

More information about the company and cA2 can be found on Centocor's home page located at the following address. For more information about Crohn's disease or ulcerative colitis, a related disorder, contact the Crohn's & Colitis Foundation of America, at 1-800-343-3637 (website: http://www.ccfa.org).

Return to News Story Page

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(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

ATCGGACGTGGACGTGCAGA 20

What is claimed is:

1. A chimeric antibody comprising at least part of a human immunoglobulin constant region and at least part of a non-human immunoglobulin variable region, said antibody capable of binding an epitope specific for human tumor necrosis factor TNFα, wherein the non-human immunoglobulin variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 3 and SEQ ID NO: 5.

2. An immunoassay method for detecting human TNF in a sample, comprising:

(a) contacting said sample with an antibody according to claim 1, or a TNF binding fragment thereof, in detectably labeled form; and

(b) detecting the binding of the antibody to said TNF.

3. A chimeric antibody comprising at least part of a human immunoglobulin constant region and at least part of a non-human immunoglobulin variable region, said antibody capable of binding an epitope specific for human tumor necrosis factor TNFα, wherein the non-human immunoglobulin variable region comprises a polypeptide encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 2 and SEQ ID NO: 4.

4. An immunoassay method for detecting human TNF in a sample, comprising:

(a) contacting said sample with an antibody according to claim 3, or a TNF binding fragment thereof, in detectably labeled form; and

(b) detecting the binding of the antibody to said TNF.

5. A chimeric antibody, comprising two light chains and two heavy chains, each of said chains comprising at least part of a human immunoglobulin constant region and at least part of a non-human immunoglobulin variable region, said variable region capable of binding an epitope of human tumor necrosis factor hTNFα, wherein said light chains comprise variable regions comprising SEQ ID NO: 3 and said heavy chains comprise variable regions comprising SEQ ID NO: 5.

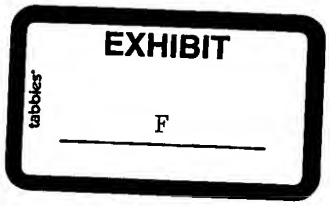
6. A chimeric antibody according to claim 5, wherein the human immunoglobulin constant region is an IgG1.

7. A chimeric antibody comprising at least part of a human IgG1 constant region and at least part of a non-human immunoglobulin variable region, said antibody capable of binding an epitope specific for human TNFα, wherein the non-human immunoglobulin variable region comprises a polypeptide encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 2 and SEQ ID NO: 4.

8. A polypeptide comprising the amino acid sequence of SEQ ID NO: 3, wherein said polypeptide binds to h TNFα and competitively inhibits the binding of monoclonal antibody cA2 to hTNFα.

9. A polypeptide comprising the amino acid sequence of SEQ ID NO: 5, wherein said polypeptide binds to h TNFα and competitively inhibits the binding of monoclonal antibody cA2 to hTNFα.

* * * * *



Brief Definitive Report

THE IMMUNOGENICITY OF CHIMERIC ANTIBODIES

By MARIANNE BRÜGGEMANN,* GREG WINTER,‡ HERMAN WALDMANN,* AND MICHAEL S. NEUBERGER‡

*From the *Division of Immunology, Department of Pathology, Addenbrooke's Hospital, Cambridge CB2 2QQ; and the ‡Medical Research Council Laboratory of Molecular Biology, Cambridge CB2 2QH, United Kingdom*

Owing to problems in making high affinity human mAbs, there is interest in the therapeutic application of chimeric antibodies in which either the V domains or just the hypervariable regions of rodent mAbs have been used to replace the equivalent parts of a human antibody (1, 2, and references therein). Whereas xenogeneic antibodies are highly immunogenic in man (see reference 3 for references), little is known about the immunogenicity of chimeric antibodies. It is unclear to what extent a particular V domain is characteristic of the species from which it originates, and therefore, whether a response will be elicited by an antibody in which only the V region is foreign. If there is such an antiidiotypic response, to what extent is it enhanced by linkage to foreign C domains? Here, we describe experiments carried out in the mouse that address these questions.

Materials and Methods

Mice and Immunizations. Mice were from Olac, Bicester, UK, or National Institute for Medical Research, Mill Hill, UK. Prebleed sera were taken from 6–8-wk-old females (six per group), which were then injected intraperitoneally with the relevant antibody (40 μg) in CFA. Serum was taken 30 d later, and the animals were boosted intraperitoneally with the same antibody (40 μg) in IFA; serum was taken after a further 10 d. For injection with cell-bound antibody, spleen cells from F_1 mice were conjugated with 4-hydroxy-3-nitrophenacetyl (NP)-kephalin (4); mice were immunized intravenously with 5×10^6 syngeneic NP-spleen cells mixed with 40 μg of anti-NP antibody. The boost (day 30) was the same as the primary immunization.

Antibodies and Immunoassays. Antibodies were purified by affinity chromatography (4) from the supernatants of cells of the J558L plasmacytoma (which secretes λ L chains) transfected with plasmids directing the synthesis of the appropriate antibody H chain. The H chain genes for HuV_{NP}-Huγ2, HuV_{NP}-Moγ2b^b, and MoV_{NP}-Moγ2b^b were assembled by inserting C_κ exon fragments (7.2-kb Hind III–Bam HI fragment for human γ2 [described in reference 4]; 4.2-kb Eco RI–Bgl II fragment for mouse γ2b^b [ref. 5]) into the pSV-V_{NP} vector or a derivative containing the HuV_{NP} V domain (2). Other transfectants have been described (1, 2, 4).

Antibody responses were measured by ELISA. Serum dilutions were incubated in microtitre plates coated with the relevant IgH, λ anti-NP antibody. Bound antiantibodies were detected using biotinylated anti-mouse κ antiserum and horseradish peroxidase coupled to streptavidin. Immune sera had less than threefold the prebleed titre of residual λ-bearing anti-NP anti-

Marianne Brüggemann was supported by a Leukemia Society of America special fellowship; her present address is the Institute of Animal Physiology and Genetics Research, Babraham, Cambridge, CB2 4AT, UK.

Address correspondence to Michael Neuberger, Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK.

body, as well as of antibodies reacting with either mouse IgM, λ myeloma protein, or purified λ L chains.

Results and Discussion

The response was compared of mice injected with one of three antibodies. The most xenogeneic antibody (HuV_{NP}-Huγ2) is composed of a human γ2 C region linked to a V domain that has the framework residues of the human NEW myeloma protein (Fig. 1). A chimeric derivative (in which only the V region frameworks are human) was created by substituting the human Cγ2 by the Cγ2b of C57BL/6 mice to yield HuV_{NP}-Moγ2b^b. In MoV_{NP}-Moγ2b^b (the syngeneic antibody), the entire V domain is of mouse origin, the foreign framework residues having been substituted by mouse sequences. All the antibodies contain a mouse λ L chain, as well as V_H hypervariable region sequences derived from a mouse antibody specific for NP.

Groups of (C57BL/6 × BALB/c)F_1 mice were immunized intraperitoneally with the three antibodies in CFA. The mice made a strong primary and secondary response to the most xenogeneic antibody, a reduced yet nevertheless considerable response to the chimeric antibody, but no detectable response to the syngeneic antibody (Fig. 2 *A*). In the mice immunized with the HuV_{NP}-Huγ2, a large proportion of the response was directed against the human γ2 C region, as witnessed by binding inhibition assays using a human IgG2 myeloma protein; much less inhibition was given by an antibody (HuV_{NP}-Huε) whose H chain is composed of the HuV_{NP} V_H domain linked to human Cε (Fig. 3 *A*, *I*). The anti-V region response elicited by the xenogeneic antibody HuV_{NP}-Huγ2 was measured using a HuV_{NP}-Huε coat; it was of a similar order to that elicited by HuV_{NP}-Moγ2b^b (Fig. 3 *B*). Thus, a considerable proportion of the response to the xenogeneic antibody was directed against the V region; this antiidiotypic response was not diminished by using the chimeric antibody with self C regions.

The antiidiotypic response in the mice immunized with either HuV_{NP}-Huγ2 or HuV_{NP}-Moγ2b^b was not exclusively directed against the human frameworks of the immunizing antibody, although these are the only foreign determinants in the V domain. The mice contained a significant titre of antibodies that recognized MoV_{NP} (Fig. 3, *A* and *B*). A more direct demonstration that it is possible to elicit an antibody response to syngeneic V domains is provided by immunizing mice with MoV_{NP}-Huγ2 (Fig. 3 *B*). Thus, the mouse can make a response to its own V domains, and probably to the hypervariable regions themselves. However, this response is not elicited unless the administered antibody contains some foreign determinants.

As a better system to mimic the use of mAbs directed against tumor cell surface

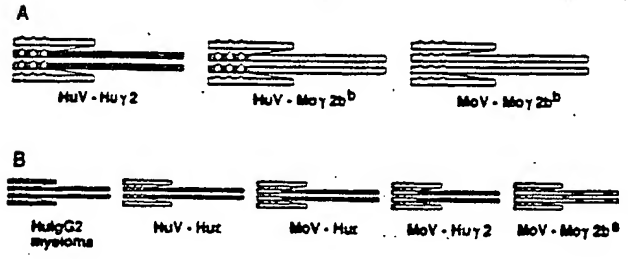
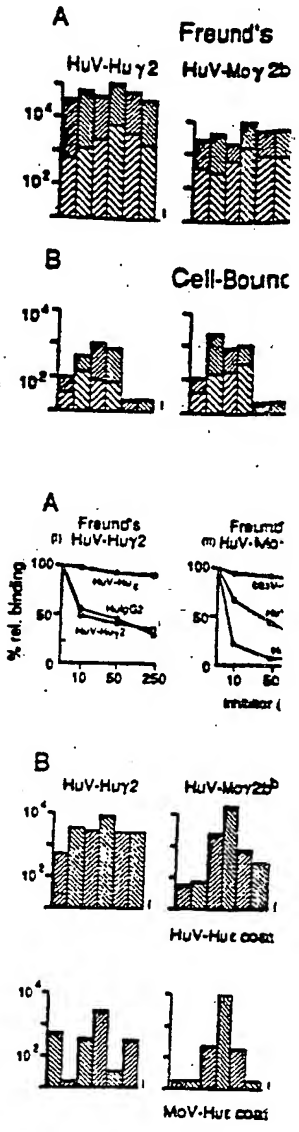


FIGURE 1. Structure of antibodies. (*A*) Antibodies used for immunization. (*B*) Antibodies used for testing the specificity of the responses. The open and filled bars denote sequences of mouse and human origin, respectively. (×) Amino acid positions at which MoV_{NP}-Moγ2b^a and MoV_{NP}-Moγ2b^b differ.



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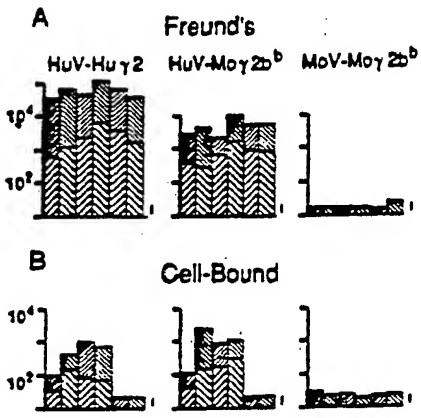

FIGURE 2. Responses to administered antibodies. (*A*) Responses to antibodies emulsified in Freund's. (*B*) Responses to antibodies bound to syngeneic spleen cells. Bars in the histogram give the serum dilution from individual mice that yield half-maximal binding to the immunizing antibody immobilized on the plate. Thus, sera from MoV_{NP}-Moγ2b^b-immunized mice were tested on an MoV_{NP}-Moγ2b^b coat, etc. Lightly crosshatched bars give titres for the primary response; stronger crosshatching indicating the increase in the secondary response. A bar indicates the titres obtained from the preimmune sera. Where there was no significant difference between the primary and secondary responses, only the secondary is depicted.

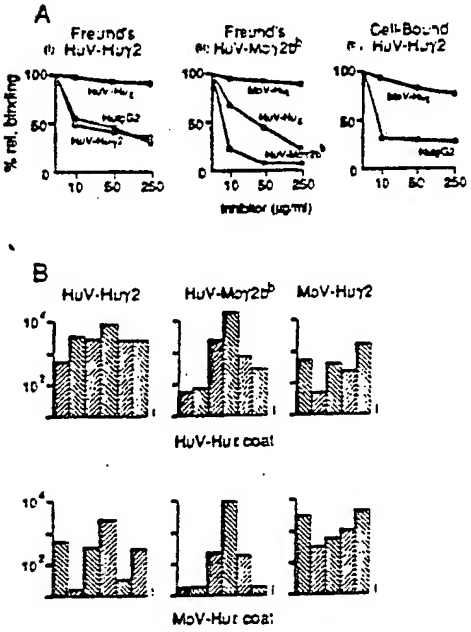

FIGURE 3. Specificity of the antiantibodies. (*A*) Binding inhibition assays. The bindings of antiantibodies in a serum dilution from individual mice hyperimmunized with (*I*) HuV_{NP}-Huγ2 in Freund's, (*II*) HuV_{NP}-Moγ2b^b in Freund's, or (*III*) cell-bound HuV_{NP}-Huγ2 were tested on a coat of the immunizing antibody in the presence of various concentrations of competitor. The result is given as the percentage binding relative to that obtained in the absence of inhibitor. Inhibition assays shown are for individual mice but are representative of the three in each group tested. (*B*) Direct binding of sera from mice hyperimmunized with HuV_{NP}-Huγ2, HuV_{NP}-Moγ2b^b, or MoV_{NP}-Huγ2 antibody in Freund's to a MoV_{NP}-Hue or HuV_{NP}-Hue coat; binding could not be inhibited with a human IgE myeloma protein. Bars for individual mice titred on MoV_{NP}-Hue are aligned with bars for the same mice titred on HuV_{NP}-Hue.

markers, mice were challenged with syngeneic spleen cells to which antibody had been bound. While the responses were considerably weaker than to the antibodies administered in CFA, the cell-bound xenogeneic and chimeric antibodies nevertheless elicited a clear response with the major part of the response to HuV_{NP}-Huγ2 being directed against the C region (Figs. 3 *A* and 2 *B*). Within the variation from individual animals, there was no clear difference in the immunogenicity of the xenogeneic and chimeric antibodies. The contrast between these results and those obtained using Freund's might be accounted for by the fact that mouse IgG2b, but not human IgG2, binds to some mouse Fc receptors (6).

Although administration of a syngeneic antibody need not elicit an antiantibody

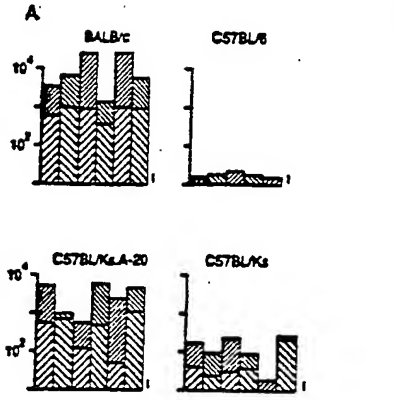

FIGURE 4. Allogeneic responses. (*A*) The response of C57BL/6, BALB/c, C57BL/Ks, and C57BL/Ks.A-20 mice to immunization intraperitoneally with MoV_{NP}-Moγ2b^b antibody in Freund's. (*B*) Binding inhibition assay of the antiantibody response in one of the MoV_{NP}-Moγ2b^b-immunized BALB/c mice. Of three other mice tested, two gave curves similar to those presented, whereas one showed a greater degree of inhibition by MoV_{NP}-γ2b^b than by MoV_{NP}-Hue.

response, polymorphism within the human population may lead to responses even to wholly human antibodies. To compare the magnitude of such an allotypic response with the response mounted against foreign V region frameworks, MoV_{NP}-Moγ2b^b was injected into both C57BL/6 (the strain from which the antibody originates) and BALB/c mice. Unlike C57BL/6, the BALB/c mice made a strong response against MoV_{NP}-Moγ2b^b, recognizing both V and C domains (Fig. 4 *A*). Although immune response genes could well play a role (7), the difference in the response obtained with the C57BL/6, BALB/c, and F_1 mice is likely to be due to the difference in Igh haplotypes. This was confirmed by comparing the responses of C57BL/Ks (H2^d, Igh^b) with C57BL/Ks.A-20 (H2^d, Igh^a) mice (Fig. 4 *B*).

Thus, an antibody with both foreign C_H domains and foreign V_H frameworks was strongly immunogenic, eliciting a response that was largely directed against the C region but with a substantial component against the V. In a chimeric derivative (in which only the V region frameworks are foreign), the anti-C response was abolished but the response to the V remained and was unattenuated. While all foreign framework sequences may not prove equally immunogenic, the results indicate that, short of administering an autologous antibody, therapeutic applications should make use of antibodies in which care has been taken to reduce the V region immunogenicity. However, the immunogenicity of antibodies in which the hypervariable regions are the sole foreign determinants is an unknown quantity and is an important focus for further research. Extrapolating to therapy in man, the results caution that, even with wholly human antibodies, problems may be encountered with allogeneic responses directed against both the V and the C. Ultimately, it may prove advisable not just to use humanized antibodies, but to use antibodies whose allotype is matched to that of the patient.

Summary

Mice were immunized with model xenogeneic (both the V_H frameworks and the C_H domains of human origin), chimeric (just V_H frameworks human), or self antibodies, and the antiantibody responses were dissected. Only the self antibody did not elicit a response. A strong response was elicited by the most xenogeneic antibody with ~90% against the C and ~10% against the V. The anti-V response was not

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attenuated in the chimeric antibody, demonstrating that foreign V_H frameworks can be sufficient to lead to a strong antiantibody response. The magnitude of this xenogeneic anti-V_H response was similar to that of the allotypic response elicited by immunizing mice of the Igh^a allotype with an Igh^b antibody. Thus, although chimerization can diminish antiantibody responses, attention should be paid both to V region immunogenicity and to polymorphism.

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Immunotherapy and other novel therapies

David E. Trentham, MD

Beth Israel Hospital, Boston, Massachusetts, USA

Although no true breakthroughs occurred, publications during the 12-month period of this review added substantial definition to certain novel immunotherapies potentially applicable to the treatment of rheumatoid arthritis. Overall, this period witnessed maturation in the field of biologic interventions. Clinical trials provided further data needed to assess the efficacy of high-dose intravenous γ-globulin therapy in patients with systemic juvenile rheumatoid arthritis, and extended uncontrolled experience with interferon-γ in adult rheumatoid arthritis was obtained. An intriguing immunostimulant and antiviral drug, isoprinosine (inosine pranobex), failed in a scientifically rigorous trial in rheumatoid arthritis. Provocative insights into totally new approaches surfaced in additional reports from a variety of immunologic areas. Although seemingly distal to rheumatoid arthritis, these papers are cited because their further development or adaptations could reach a stage where clinical trials in rheumatoid arthritis are warranted.

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Intravenous gammaglobulin therapy

Diseases for which high-dose intravenous γ-globulin therapy might be effective continue to proliferate. In a pilot study, seven of eight patients with treatment-resistant active juvenile rheumatoid arthritis, including systemic features, responded to a regimen similar to that in standard use for idiopathic thrombocytopenic purpura and other autoimmune disorders [1••]. Although open in design, most of the clinical outcome variables measured changed substantially, lending credibility to a conclusion that the intervention did indeed modify the disease. Improvement in fairly objective parameters, including the erythrocyte sedimentation rate and hemoglobin and albumin levels, were noted as well. The major problems with intravenous γ-globulin therapy seem to be monetary and logistic. Although the mechanism(s) responsible for the salutary effect remains elusive, additional evaluations of intravenous γ-globulin therapy in juvenile rheumatoid arthritis clearly appear to be indicated.

Pulse methylprednisolone or nitrogen mustard therapy

Although practiced not infrequently by certain experienced clinical rheumatologists, there is little literature on pulse treatment with methylprednisolone or nitrogen mustard. Thus, the review from the Cleveland Clinic Foundation is of importance [2•]. Both methodologic details and clinical and immunologic sequelae of high-dose intravenous pulse steroid and chemotherapy are detailed in a nonjudgmental fashion in this article. Based on their experience and the data displayed in the independent reports cited, the efficacy to toxicity ratio of nitrogen mustard appears to be acceptable for patients whose rheumatoid arthritis is refractory to more conventional approaches. The ready availability of mustard to rheumatologists would seem to justify further attention to this relatively neglected area of therapeutics, if a truly scientific format is followed.

Recombinant interferon-γ

Subcutaneous administration of recombinant interferon-γ as a potential treatment for rheumatoid arthritis has exhibited a discordant record in clinical studies to date. All uncontrolled trials have been short term, and although improvement has been described, benefit occurred in only two of the four placebo-controlled studies. Thus, the extended, open-label, 2-year follow-up of patients who participated in a prospective double-blind US multicenter trial is of interest [3•]. At 1- and 2-year follow-ups, 57% and 38% of the patients, respectively, remained on the agent. Improvement, compared to study entry, was evident in perhaps one third of the patients at 1 year and in 15% to 20% at 2 years. Systemic side effects (fever, chills, headache, nausea, diarrhea, and local reactions at the injection site), so troublesome in the short-term studies, seemed to abate, with only fever being a significant problem.

Unfortunately, plaudits stop here. A number of major weaknesses make the study extremely difficult to interpret. Concurrent therapy with a gamut of drugs was permitted; the majority of patients were receiving nonsteroidal anti-inflammatory drugs, prednisone, and one of several so-called remittive regimens (gold, penicillamine, hydroxychloroquine, and even gold plus hydroxychloroquine). Even more confounding is the variable decrease in dose and frequency of interferon-γ that these patients received. It is likely that less exposure to the agent explains the better "tolerability" to the therapy. It is much more difficult to identify the drugs that were responsible for the improvement in this cohort.

Isoprinosine fails

The past decade has seen a series of putative immunoenhancer and antiviral agents surface as dualistic approaches for the curtailment of rheumatoid arthritis. Levamisole and amiprilose were two of the more widely touted drugs of this genre. During this period, another candidate known by a variety of names, including isoprinosine, inosine pranobex, inosiplex, and even methisoprinol slowly matured along this pathway. Lymphocyte stimulatory and antiviral properties were defined, but unfortunately most of the work was outside of the boundaries of peer-reviewed journals. Purportedly beneficial effects in rheumatoid arthritis appeared a number of years ago in an open study, prompting a careful, large-scale scrutiny within a double-blind, placebo-controlled format at the prestigious Center for Rheumatic Disease in Glasgow [4•]. Unfortunately, no significant improvement in any disease variable was noted in this 24-week trial. Equally intriguing was the fact that no evidence of spontaneous improvement in the disease occurred in the placebo limb, unlike the response to placebo in several recent trials in the United States. Are the Scots less suggestible, or has the so-called "placebo effect" been exaggerated recently?

Misoprostol: a gastroenterologic bequeathment to rheumatologists?

Pioneering, but often overlooked, work performed two decades ago elucidated striking anti-inflammatory properties for prostaglandins at pharmacologic doses in the adjuvant model of arthritis. In a variety of contexts, additional tantalizing properties for prostaglandin analogues have surfaced and engendered interest in their potential role in the treatment of immunologically mediated inflammatory arthritis [5•]. The most extensively studied candidate is misoprostol, the prostaglandin E_1 analogue now widely used to thwart nonsteroidal anti-inflammatory drug–induced gastropathic bleeding. An additional impetus to this theory has been several disclaimers of the "Vane hypothesis," *ie*, that prostaglandins are the major inciters of inflammation and their attenuation explains the efficacy of nonsteroidal anti-inflammatory drugs in inflammatory arthritis. Now that the precise mechanism of action of nonsteroidal anti-inflammatory drugs seems to have fallen into a "black hole," the once-antithetical possibility that prostaglandins might be clinically useful anti-inflammatory drugs is being considered. How this might be accomplished is a mystery. Could high ambient levels of prostaglandins inhibit macrophage or polymorphonuclear leukocyte functions, such as chemotaxis? Alternatively, could this feat be accomplished by immunoregulatory actions? *In vitro*, misoprostol has been reported to suppress lymphocyte proliferative responses. *In vivo*, misoprostol administration promoted the ability of humans to retain renal allografts. Further exploration into the possibility that prostaglandin E_1 analogues alter T-cell processes could bring about a new therapeutic dimension for patients with rheumatoid arthritis far beyond their action on the gastric mucosa.

Biologic inhibitors

The new frontier of intervention into autoimmune disease by specific, targeted biologic products (the plentitude, purity, and specificity of which are tightly insured by production via recombinant technology) continues to emerge. Potential applications, including osteoarthritis as well as rheumatoid arthritis, were recently reviewed by Hess [6•]. In terms of actual development, recent media attention has been devoted largely to a recombinant protein able to specifically inhibit the alleged central culprit in rheumatoid arthritis: interleukin-1. Paralleling this effort has been the purification of a natural inhibitor of another cytokine, tumor necrosis factor [7•]. Although the relevance of tumor necrosis factor and the biologic outcome of its banishment by a monospecific inhibitor remain in doubt, the isolation of interleukin inhibitors strengthens the probability that interleukin-mediated processes all involve precise cell surface receptors, and abrogation of the activity can be achieved by intervening with either the factor or its surface receptor.

The recent elucidation of a contra-interleukin-2 cytokine in the mouse [8•] further illustrates this theme. Based on the importance of T cells and probably interleukin-2 in the pathogenesis of the collagen model of rheumatoid arthritis, clones of T cell from collagen-immunized mice were probed and some were found to secrete a factor that specifically antagonized interleukin-2-mediated pathways *in vitro*. Clinical assessment in the model revealed that inflammation could be reduced by injecting this protein. Although the exact biochemical nature of this adversarial product is uncertain, extrapolation of this approach to the future treatment of rheumatoid arthritis can be envisioned.

Another report [9•] interjected caution into these approaches. While specific biologic antagonists for the immune system are being designed, often in "high-tech" fashion, naturally occurring counterparts are being elucidated as well. The existence of these naturally occurring contra molecules could argue that treatment with "designer molecules" would be superfluous and therefore ineffective. The recognition that autoantibodies of the IgG isotype capable of neutralizing the activity of interleukin-1 can be found in the sera of some patients with rheumatoid arthritis [9•] would argue that in certain stages of the disease, the body has already at least partially checkmated the process. Thus, additional therapeutic intervention would be biologically effete. Unidimensional attacks on aberrant immune pathways might have a limited effect on the underlying disease process.

Another example of a highly targeted approach is the use of a novel recombinant fusion protein with impressive specificity for the high-affinity interleukin-2 receptor. DAB_{486} interleukin-2 is a conjugate consisting of a portion of interleukin-2 joined to a diphtheria toxin fragment. This amalgam enables the hybrid species to interact as a ligand with the cell surface receptor for interleukin-2 and then deliver a lethal hit. Activated T cells express the interleukin-2 receptor, causing the molecule to be therapeutically appealing. Recent evaluation in adjuvant arthritis indicated that use of the protein was capable of suppressing both the induction and established stage of this model [10•]. By inference, these data strongly imply that interleukin-2-positive cells orchestrate a major portion of the pathogenesis of this disease. A theoretic concern with the use of this diphtheria conjugate to treat autoimmune disease in humans is their ubiquitous prior exposure to diphtheria vaccines, *ie*, that circulating antibodies to diphtheria might latch on and capture the immunologic missile before it could encounter the target cells. To address this potential liability, rats were preimmunized to diphtheria before the attempted induction of adjuvant arthritis. Even in these rats, where appreciable titers of diphtheria antibodies were found, the interleukin-2 receptor immunotoxin was effective. Inceptual feasibility work in rheumatoid arthritis was begun, and additional information regarding prospects for this approach should soon be available.

Hypothetical vaccination strategies also reside within the realm of targeted biologic therapies. Candidate autoantigens have been identified, including type II collagen and heat shock protein in rheumatoid arthritis. Schemes by which antigen-specific immunosuppression can be achieved have also been established, such as intravenous T-line cell inoculation. Earlier work showed that intravenous injection of antigen-coupled mononuclear cells or erythrocytes could abort an immune response to the antigen. Type II collagen coupled cells, injected intravenously, were used to attenuate the onset of adjuvant arthritis, demonstrating that intrinsically similar pathways operate in both the collagen and adjuvant models. Practicality, however, was lacking in this approach. More recently, investigators have unearthed another approach harkening back to older literature that cited peroral allergen administration as capable of expurgating atopic responses. For example, ingestion of poison ivy was claimed to be a way to alleviate contact sensitivity to this stimulus. The basic tenant is that gut lymphoid tissue is preferentially primed towards suppression of immune responses. Recent work has shown that experimental allergic encephalomyelitis and collagen arthritis can be prevented by peroral administration of myelin basic protein and type II collagen, respectively. Innovative experiments by Zhang *et al.* [11••] have shown that type II collagen ingestion can also downregulate adjuvant arthritis, duplicating the earlier results with collagen-coupled cells. Additional experiments revealed that immunosuppression was antigen specific and capable of being adoptively transferred with cells that were probably T suppressor in nature [11••]. The safety and simplicity of this vaccination protocol have been sufficiently compelling to warrant study in humans, seeking to ascertain whether curtailment of autoimmune disease in humans can be achieved by this approach. Major unanswered questions at present include 1) whether the human immune system recapitulates that of the mouse and therefore can be downregulated by oral antigen delivery, and 2) whether an established immune process, as is operative in autoimmune disease, can be quiesced by oral antigen intake.

Ideas for potential biologic approaches do not come solely from molecular biology laboratories. Harris and Sledge [12•] recently pinpointed in print the intriguing phenomenon that artificial hip replacement in patients with rheumatoid arthritis consistently results in sustained abatement of inflammation in the joint, whereas a similar outcome is not evident with knee replacement. In their opinion, the major differences in the two procedures relate to the complete absence of residual cartilage after a hip replacement, whereas patellar cartilage remains in a total knee joint replacement. Does the lack of cartilage explain the alleviation of synovitis after hip replacement? This experience seems sufficiently provocative to warrant further scrutiny into the precise nature of the provocative material in cartilage. Is it collagen? Is it proteoglycan? Is it possibly even a sequestered infective agent or by-product?

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Possibly an important clue furnished by surgeons who keep their eyes open and minds engaged.

David E. Trentham, MD, Chief, Division of Rheumatology, Beth Israel Hospital, 330 Brookline Ave., Boston, MA 02215, USA.

CURRICULUM VITAE

Jan T. Vilcek

Home Address: 920 Fifth Avenue, New York, NY 10021

Date and Place of Birth: June 17, 1933; Bratislava, Czechoslovakia

Education:

Comenius University Medical School, Bratislava; M.D., 1957

Institute of Virology, Czechoslovak Academy of Sciences, Bratislava; C.Sc. (equivalent to Ph.D.), 1962

Professional Positions and Appointments:

1973 - present	Professor of Microbiology New York University School of Medicine
1983 - present	Head, Cytokine Research Unit, New York University School of Medicine
2000 - present	Microbiology Course Director New York University School of Medicine
1987 - 1997	Co-Director, Cancer Center Core Clinical (BRM) Laboratory, New York University Medical Center
1984-1993	Director, Microbiology Graduate Training Program, New York University School of Medicine
1968-1973	Associate Professor of Microbiology New York University School of Medicine
1968-1973	US Public Health Service Research Career Development Award
1965-1968	Assistant Professor of Microbiology New York University School of Medicine
1962-1964	Head of Laboratory, Institute of Virology Czechoslovak Academy of Sciences, Bratislava
1959-1962	Fellow of the Czechoslovak Academy of Sciences, Bratislava
1957-1959	Research Associate Inst. of Virology, Czechoslovak Academy of Sciences, Bratislava

Honors:

Recognition Award, Japanese Inflammation Society, 1989
Outstanding Investigator Grant, National Cancer Institute, 1991
Elliott Osserman Award in Cancer Research, 1996
Fellow of the American Association for the Advancement of Science, 1997
Distinguished Alumnus Award and Medal, Comenius University, Bratislava, 2001
Biotechnology Achievement Award, NYU School of Medicine, 2002
Honorary Lifetime Membership Award of the International Cytokine Society, 2003
Honorary Membership, International Society for Interferon & Cytokine Research, 2003
Included in ISIHighlyCited among 250 most highly cited authors in Immunology category
Presidential Citation of New York University for contributions to NYU Medical School, 2004

Editorial Activities:

Editor-in-Chief, Archives of Virology, 1975-1984
Associate Editor, Archives of Virology, 1985-1991
Associate Editor, Virology, 1977-1979
Associate Editor, Interferon (Academic Press), 1979-1988
Associate Editor, Journal of Interferon and Cytokine Research, 1980-present
Associate Editor, Applied Biochemistry and Biotechnology, 1981-1986
Associate Editor, Infection and Immunity, 1983-1985
Associate Editor, Antiviral Research, 1984-1988
Associate Editor, Natural Immunity and Cell Growth Regulation, 1985-1992
Associate Editor, Journal of Immunological Methods, 1986-present
Associate Editor, Journal of Immunology, 1987-1989
Associate Editor, Lymphokine and Cytokine Research, 1987-1994
Advisory Editorial Board member, ISI Atlas in Science: Immunology, 1987-1989
Editorial Board member, Journal of Biological Chemistry, 1988-1990
Section Editor, Aging: Immunology and Infectious Disease, 1988-1995
Editorial Board member, Journal of Cellular Physiology, 1988-present
Advisory Editorial Board member, Cytokine, 1989-present
Editorial Board member, Biologicals, 1989-1995
Editorial Board member, Acta Virologica, 1991-present
Associate Editor, International Archives of Allergy and Immunology, 1992-1997
Editorial Board member, Cellular Immunology, 1992-1996
Editorial Board member, Folia Biologica (Prague), 1993-present
Contributing Editor, Journal of Inflammation, 1994-1998
Editor-in-Chief, Cytokine and Growth Factor Reviews, 1995-present
Editorial Board member, Cytokines, Cellular & Molecular Therapy, 1998-present

Selected National and International Committees:

American Cancer Society Advisory Committee on Microbiology and Virology, Member 1981-1984; Chairman 1984

American Cancer Society Advisory Committee on Interferon, 1984-1988

WHO Committee on Interferon Nomenclature, Member 1979-1985, Chairman 1981-1985

WHO Consultant on biological standardization, 1978-1989

Scientific Advisory Board, Max-Planck-Institute for Biochemistry, Munich, German Federal Republic, 1987-1995

International Advisory Board, Czech Immunological Society, 1991-present

Member, American Heart Association Fellowship Review Committee, 1992-1994

Member, Israel Cancer Research Fund Scientific Review Panel, 1993-1996

National Cancer Institute Cancer Center and Research Programs Review Committee (Subcommittee C), 1994

National Cancer Institute Scientific Review Group, Subcommittee C, 1997

Morehouse School of Medicine/ Univ. of Alabama Cancer Center External Advisory Committee, 2001-present

Recent Medical School and University Committees:

President's Committee on Sponsored Research
University Committee on Institutional Responsibility
University Confidentiality Issues Committee
Medical School Technology Transfer and Patents Committee
General Clinical Research Center Executive Advisory Committee
School of Medicine Grievance Committee
School of Medicine Committee on Conflict of Interest (Chairman)
Center for AIDS Research Advisory Board

Membership in Professional Societies:

American Society for Microbiology
American Association for the Advancement of Science (Fellow)
American Association of Immunologists
International Society for Interferon and Cytokine Research
Czech Immunological Society
International Cytokine Society (President, 1997-98)
Czechoslovak Society for Microbiology

Books Edited:

Regulatory Functions of Interferon. New York Academy of Sciences, 1980 (edited with T.C. Merigan and I. Gresser)

The Clinical Potential of Interferons. University of Tokyo Press, 1982 (edited with R. Kono)

Interferons and the Immune System. Elsevier, 1984 (edited with E. De Maeyer)

Tumor Necrosis Factors: Structure, Function, and Mechanisms of Action. Marcel Dekker, Inc., 1991 (edited with B.B. Aggarwal)

Cytokine Reference. Academic Press, 2000 (edited with J.J. Oppenheim, M. Feldmann *et al.*)

Major Conferences Organized:

New York Heart Association Symposium on Interferon, New York, 1969

New York Academy of Sciences Conference "Regulatory Functions of Interferons", New York, 1979 (co-chaired with Ion Gresser and Thomas C. Merigan)

Congress on Cytokine Research, Boston, 1986 (co-chaired with Stanley Cohen)

Second Congress on Cytokine Research and Growth Factors, Philadelphia, 1987 (co-chaired with Stanley Cohen and Renato Baserga)

Seventh International Lymphokine Workshop, San Antonio, 1990 (co-chaired with Lawrence Lachman and William Farrar)

Seventh Annual Conference of the International Cytokine Society, Hilton Head, SC, 1999 (co-chaired with Bruce Beutler, Scott Durum and Ann Richmond)

Cytokine-Regulated Gene Expression at the Crossroads of Innate Immunity, Inflammation and Fertility. New York, NY, 2003 (co-chaired with Bruce Cronstein and Tibor Glant)

Current Teaching Activities:

Microbiology Course for medical and graduate students (Course Director and lecturer)
Immunology Course for medical students (lecturer)
Immunology Course for graduate students (lecturer)
Foundations in Cell and Molecular Biology Course for graduate students (lecturer)

Graduate Students and Fellows:

Ph.D. thesis advisor to 29 students who completed their doctorates between 1971-2000.

Past students and fellows include: Toby Rossman, Mun H. Ng, Douglas R. Lowy, Brian Berman, Masayoshi Kohase, Edward A. Havell, Shudo Yamazaki, Paul Anderson, Teresa G. Hayes, Masafumi Tsujimoto, Rena Feinman, Vito J. Palombella, Jian-Xin Lin, Yihong Zhang, Luiz F. L. Reis, Tae Ho Lee, Jedd D. Wolchok, Gene W. Lee, Peter J. Sciavolino, Ryutaro Kamijo, Igor C. Oliveira, Deborah Shapiro, Lidija Klampfer, Anne Altmeyer, Ilja Vietor, Paul Schwenger, John Gerecitano, Adam R. Goodman, David M. Poppers and Deborah Alpert.

U.S. Patents:

4,460,685	Method of enhancing the production of human γ interferon
4,666,865	Immunoassay for biologically active human interferon-gamma employing unique monoclonal antibodies
4,835,256	Human gamma interferon polypeptide having glutamine as the ninth n-terminal amino acid
5,386,013	Tumor necrosis factor-induced protein TSG-6
5,426,181	DNA encoding cytokine-induced protein, TSG-14
5,656,272	Methods of treating TNF-α-mediated Crohn's disease using chimeric anti-TNF antibodies
5,698,195	Methods of treating rheumatoid arthritis using chimeric anti-TNF antibodies
5,846,763	DNA encoding tumor necrosis factor stimulated gene 6 (TSG-6)
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